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ERRATUM

Through error the name of Dr. D. A. Todd appears in the personnel of the Executive Committee as given on the Journal cover instead of that of Dr. L. C. Todd as given in the Roster.

This error has been corrected with apologies to both.

ERRATUM

In the article by B. S. Kline, Control Antigen Emulsions for Microscopic Slide Precipitation Tests for Syphilis, appearing in the November, 1937 issue, in protocol 2, the word emulsions should appear as a heading over the columns relating to methods of preparation in the protocol.

ERRATUM

Through an unfortunate oversight, the contents of the September issue were not listed in the Index for Volume 7.

The page on which they appear in this issue is to be inserted in the Volume 7 index.

VACCINATION AGAINST EXPERIMENTAL MENINGOCOCCUS MENINGITIS*

JOHN A. KOLMER

WITH THE ASSISTANCE OF ANNA M. RULE

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During the past twenty-five years various investigators[1–8] have attempted to determine the value of vaccination of human beings against epidemic meningococcus meningitis. Antibody studies and especially agglutination tests, have generally indicated the production of some humoral response and in some instances it has appeared that the immunization of large groups may have contributed to the limitation of epidemics. The general results, however, have been indefinite and inconclusive, part of the difficulties encountered being due to the multiplicity of types of meningococci and particularly to the need of a test for susceptibility for aiding in the selection of individuals for active immunization as the attack rate is low.

According to Ferry, Norton and Steele[9], Branham and Lillie[10], Sickles[11] and Krestownikowa, Belkina, Dosser and Lasowsky[12] virulent meningococci may produce a soluble or extracellular toxin in broth cultures under suitable technical conditions which Ferry and Steele[13] and Kuhns[14] have found will produce positive reactions upon intracutaneous injection in about 35 to 50 per cent of presumably susceptible human beings and especially children. Furthermore, Ferry and Steele have reported that three to four subcutaneous injections of this toxin to 232 individuals of 12 to 18 years of age yielding positive skin reactions, resulted in the production of sufficient antitoxin to yield negative reactions in 62.4 to 74.6 per cent when retested about two months after the last dose, while Kuhns reported 72 to 84 per cent negative reac-

* Read before the American Society of Clinical Pathologists, Philadelphia, June 4, 1937. Received for publication June 6th, 1937.

tions among a group of 316 positive reactions when tested several months after subcutaneous injections of the toxin.

Under the conditions it would appear that part at least of the virulence and pathogenicity of meningococci may be due to a true extracellular toxin which, according to Ferry and Shormack[15], when injected intracisternally is capable of producing in laboratory animals, and especially monkeys, symptoms in nature and severity approaching those following injections of live meningococci. But whether or not the presence in the blood of sufficient natural or acquired antitoxin to yield negative skin reactions is indicative of a state of effectual resistance to meningococcus infections, remains to be more definitely determined.

If, however, this skin test is ultimately proven to be of value for detecting susceptibility to the meningococcus among human beings, great encouragement would be given efforts toward vaccination against meningitis and especially if it were found effective against experimental meningococcus meningitis of the lower animals. For these reasons we have thought it advisable to determine the value of vaccination against experimental meningitis of guinea pigs, rabbits and monkeys, since it does not appear that sufficiently extensive investigation of this phase of the subject has been hitherto reported.

EXPERIMENTAL

Cultures of Meningococci. Considerable difficulty was experienced in securing strains capable of producing severe meningitis in guinea pigs, rabbits and especially monkeys by intracisternal and intraspinal inoculation, since the plan of our study was to determine if vaccination was capable of not only producing agglutinins and complement fixing antibody, but of protecting these animals against experimental meningococcus meningitis by these routes of inoculation.

After preliminary virulence tests with many different cultures, consisting of the intracisternal inoculation of young guinea pigs, rabbits and monkeys, we decided to use a culture of Type I (Gordon) meningococcus kindly furnished by Dr. N. S. Ferry, and one of Type III (Gordon) secured in cultures of the blood and cerebrospinal fluid of a rapidly fatal case of meningitis occurring in a child in Temple University Hospital. These cultures were maintained on blood agar and while their virulence fluctuated we were successful in maintaining sufficiently high virulence by frequent intracisternal inoculations of rabbits and guinea pigs.

Guinea pigs (300–450 grams) inoculated intracisternally with 0.15 c.c. of suspension of 36-hour blood agar cultures in saline solution (0.3–0.45 c.c. per

kilogram) succumbed in 24 to 72 hours with severe meningitis; about 75 per cent of rabbits (1200–2400 grams) inoculated intracisternally with 1 c.c. of the suspensions (0.25–0.5 c.c. per kilogram) succumbed to severe meningitis, but monkeys (2100–4200 grams) inoculated intraspinally with 2 c.c. (0.4–1.0 c.c. per kilogram), while developing moderately severe meningitis, have always recovered. This was true of both types so that in these experiments the meningococci were most virulent for guinea pigs, slightly less so for rabbits and still less for adult monkeys. Furthermore, intraspinal inoculation of 4 monkeys

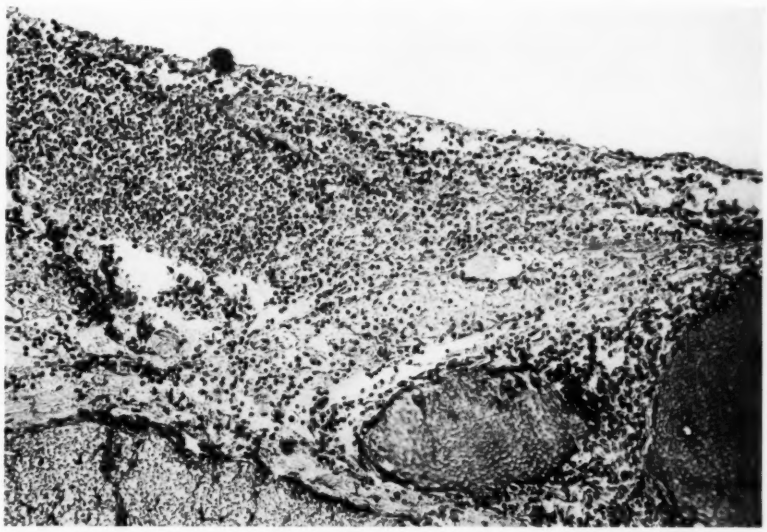

FIG. 1. Section of upper end of spinal cord and meninges of rabbit succumbing 72 hours after the intracisternal injection of saline suspension of 36-hour blood agar culture of Type I meningococcus. Acute hyperemia and edema with polymorphonuclear leukocytic infiltration.

with suspensions in sterile 6 to 10 per cent mucin in saline solution did not appear to appreciably increase the virulence of our strains.

Symptoms of meningitis usually began within four to eight hours after intracisternal inoculation and at the end of twenty-four hours cerebrospinal fluids obtained by cisternal puncture were cloudy, due to the presence of polymorphonuclear pus cells, with increased protein and showing numerous meningococci in direct smears and cultures. Sections of the brain, cord and meninges of guinea pigs and rabbits succumbing in about 72 hours after inoculation revealed a true suppurative meningitis, most marked in the upper cord and at the base of the brain, characterized by hyperemia with extensive polymorphonuclear infiltration (Fig. 1). Whether or not the experimental meningitis was produced

predominantly by the toxins, and death the result of toxemia, we are not prepared to state as all of our experiments were conducted by intracisternal or intraspinal inoculation of suspensions of 36-hour live cultures in saline solution, instead of with bouillon filtrates.

Cultures of Types II and IV (Gordon) available to us were insufficiently virulent for these animals by intracisternal inoculation and consequently were not employed.

Preparation and Administration of Vaccines. Each strain was cultivated in hormone broth at 37°C. for five days, during which time pellicles were formed. The cultures were then well shaken, diluted with sterile saline to contain approximately 100,000,000 meningococci per c.c. and tricresol added to 0.5 per cent. Each suspension was then incubated at 37°C. for twenty-four hours, cultured for sterility and kept at 4–6°C.

By this method of preparation it is likely that some of the meningococci had undergone autolysis so that dilutions to 100,000,000 per c.c. were only approximately correct, but we hoped that vaccine prepared in this manner would incorporate autolysed as well as whole chemically killed cocci along with any extracellular toxins produced during the period of 5 days of incubation, which were probably converted into toxoid by the tricresol employed.

Each vaccine was administered by subcutaneous injection to guinea pigs, rabbits and monkeys in dose of approximately 100,000,000 meningococci per kilogram of weight every 5 days for 3, 4 or 5 inoculations.

The guinea pigs varied in weight from 300 to 450 grams, the rabbits from 1500 to 2400 grams and the monkeys (*Macacus rhesus*) from 2100 to 4700 grams.

The dose employed was purposefully large corresponding to as much as 600,000,000 cocci per 60 kilograms of body weight, but we have thought that effective vaccination would require the administration of relatively large doses and the work of Gates (6) has indicated that they are well borne by human beings.

Animals have also been immunized with a vaccine of equal parts of the two suspensions of Types I and III meningococci to determine the value of mixed vaccine in the production of antibody and induced resistance against intracisternal and intraspinal inoculations with virulent organisms.

Agglutination and Complement Fixation Tests. The sera of all animals were tested for homologous agglutinins before immunization and again about four weeks after the last dose of vaccine. These tests were conducted with unheated sera and suspensions of the meningococci in saline solution prepared of 36-hour agar cultures corresponding to barium-sulphate standard No. 3 (approximately 2000 million per c.c.). The mixtures of 0.5 c.c. of varying dilutions of serum and 0.5 c.c. of bacterial suspensions were incubated at 55°C. for 4 hours and placed in the refrigerator over night when the readings were made.

Complement fixation tests were conducted only with the sera of the monkeys before and after immunization since these do not yield the non-specific reactions

so commonly observed with rabbit sera (16). The antigens were simple suspensions of 36-hour blood agar cultures in saline solution, heated at 60°C. for one hour and preserved with 0.5 per cent tricresol. Each antigen was used in a dose corresponding to one-third of its anticomplementary unit and the tests conducted according to the technic of the Kolmer modification of the Wassermann reaction (17) with varying amounts of serum.

Tests for Acquired Resistance to Meningitis. About four weeks after the last dose of vaccine the guinea pigs, including controls, were inoculated intracisternally* with 0.15 c.c. of a suspension of 36-hour blood agar culture of homologous strain of meningococcus in saline solution corresponding to about 1000 million per c.c. This dose produced fatal meningitis among all of the unvaccinated controls in 24 to 72 hours with both strains, Type III being somewhat more virulent than Type I.

The rabbits were also inoculated intracisternally with 1 c.c. of the same suspensions and produced severe meningitis among all unvaccinated controls resulting in a death rate of about 75 per cent in the case of both types.

The monkeys were inoculated intraspinally with 2 c.c. of the same suspensions according to the technic of Flexner (18). Both strains in the doses employed were apparently less virulent for these animals than for the guinea pigs and rabbits. Meningitis occurred among all of the unvaccinated controls but all of the animals finally recovered.

RESULTS

With Guinea Pigs. As shown in table 1, all of 8 guinea pigs given 3 to 5 subcutaneous injections of vaccine of Type I meningococcus showed the production of agglutinin, the amounts being somewhat higher in the case of those receiving 5 doses. Following intracisternal inoculation with virulent culture, all of 4 unvaccinated controls developed severe symptoms and succumbed in 24 to 72 hours. Of the four given 5 doses of vaccine, 2 recovered while of the four given 3 doses, one recovered.

Less encouraging results however, were observed with the eight animals given a vaccine of Type III meningococcus (table 2). All produced agglutinins but in general terms not to the same degree as produced by the Type I vaccine and all but one (received 5 doses), including 4 unvaccinated controls, succumbed in 24 to 48 hours after intracisternal inoculation.

Six animals were given 5 doses of the mixed vaccine. All

* All intracisternal and intraspinal inoculations were made under full ether anesthesia.

TABLE 1

RESULTS OF VACCINATION OF GUINEA PIGS WITH TYPE I MENINGOCOCCUS

ANIMAL NUMBER	VACCINE	AGGLUTINATION REACTIONS		RESULTS OF INTRACISTERNAL INOCULATION‡
		Before vaccination	After vaccination	
5F	5 doses*	−†	1:40	Severe symptoms; died 24 hours
6F	5 doses	−	1:80	Slight symptoms; recovered
7F	5 doses	−	1:160	Slight symptoms; recovered
8F	5 doses	−	1:40	Severe symptoms; died 36 hours
19F	3 doses	−	1:40	Severe symptoms; died 24 hours
20F	3 doses	−	1:10	Severe symptoms; died 36 hours
21F	3 doses	−	1:80	Severe symptoms; died 36 hours
22F	3 doses	−	1:20	Slight symptoms; recovered
Control	0	−	0	Severe symptoms; died 36 hours
Control	0	−	0	Severe symptoms; died 24 hours
Control	0	−	0	Severe symptoms; died 24 hours
Control	0	−	0	Severe symptoms; died 72 hours

* 100,000,000 per kilogram subcutaneously every 5 days.

† No agglutination in final dilutions of 1:10.

‡ With 0.15 c.c. of suspension of 36-hour blood agar culture in saline solution.

TABLE 2

RESULTS OF VACCINATION OF GUINEA PIGS WITH TYPE III MENINGOCOCCUS

ANIMAL NUMBER	VACCINE	AGGLUTINATION REACTIONS		RESULTS OF INTRACISTERNAL INOCULATION‡
		Before vaccination	After vaccination	
1W	5 doses*	−†	1:40	Severe symptoms; died 24 hours
2W	5 doses	−	1:40	Severe symptoms; died 48 hours
3W	5 doses	−	1:40	Severe symptoms; recovered
4W	5 doses	−	1:80	Severe symptoms; died 72 hours
15W	3 doses	−	1:40	Severe symptoms; died 24 hours
16W	3 doses	−	1:10	Severe symptoms; died 48 hours
17W	3 doses	−	1:10	Severe symptoms; died 24 hours
18W	3 doses	−	1:20	Severe symptoms; died 24 hours
Control	0	−	0	Severe symptoms; died 24 hours
Control	0	−	0	Severe symptoms; died 24 hours
Control	0	−	0	Severe symptoms; died 24 hours
Control	0	−	0	Severe symptoms; died 24 hours

* 100,000,000 per kilogram subcutaneously every 5 days.

† No agglutination in final dilutions of 1:10.

‡ With 0.15 c.c. of suspension of 36-hour blood agar culture in saline solution.

produced some agglutinins but of 3 inoculated intracisternally with Type I, one recovered while all 3 inoculated with Type III succumbed (table 3).

Six additional animals given 3 doses of the mixed vaccine produced some agglutinins but all succumbed in 24 to 72 hours when inoculated intracisternally.

TABLE 3

RESULTS OF VACCINATION OF GUINEA PIGS WITH A MIXED VACCINE OF EQUAL PARTS OF TYPES I AND III MENINGOCOCCUS

ANIMAL NUMBER	VACCINE	AGGLUTINATION REACTIONS		RESULTS OF INTRACISTERNAL INOCULATION‡	
		Before vaccination	After vaccination	With Type I	With Type III
9M	5 doses*	—†	1:80		Died 24 hours
10M	5 doses	—	1:40		Died 36 hours
11M	5 doses	—	1:40		Died 24 hours
12M	5 doses	—	1:20	Died 24 hours	
13M	5 doses	—	1:80	Recovered	
14M	5 doses	—	1:40	Died 48 hours	
23M	3 doses	—	1:40		Died 24 hours
24M	3 doses	—	1:40		Died 24 hours
25M	3 doses	—	1:20		Died 24 hours
26M	3 doses	—	1:40	Died 72 hours	
27M	3 doses	—	1:10	Died 24 hours	
28M	3 doses	—	1:80	Died 24 hours	
Control	0	—	0		Died 24 hours
Control	0	—	0		Died 24 hours
Control	0	—	0	Died 24 hours	
Control	0	—	0	Died 36 hours	

* 100,000,000 per kilogram subcutaneously of equal parts of Types I and III meningococcus every 5 days.

† No agglutination in final dilutions of 1:10 with an antigen of equal parts of Types I and III meningococci.

‡ With 0.15 c.c. of heavy suspension of 37-hour blood agar culture in saline solution.

As young guinea pigs are particularly susceptible to the meningococcus, probably more encouraging results would have been observed by inoculating intracisternally with smaller amounts of culture, but we wished to employ a dose fatal for all unvaccinated controls. In general terms, the results have indicated however,

that it is possible to actively immunize these very susceptible animals and especially against Type I meningococcus.

With Rabbits. As shown in table 4, all of eight animals immunized with the Type I vaccine showed agglutinin for the homologous strain, the four receiving five doses somewhat more than the four given three doses (table 4).

Of four unvaccinated controls inoculated intracisternally with 1 c.c. of suspension of living organisms, three succumbed in 24

TABLE 4

RESULTS OF VACCINATION OF RABBITS WITH TYPE I MENINGOCOCCUS

RABBIT NUMBER	VACCINE	AGGLUTINATION REACTIONS		RESULTS OF INTRACISTERNAL INOCULATION‡
		Before vaccination	After vaccination	
5F	5 doses*	−†	1:40	Severe symptoms; recovered
6F	5 doses	−	1:80	Slight symptoms; recovered
7F	5 doses	−	1:40	Severe symptoms; died 48 hours
8F	5 doses	−	1:160	Slight symptoms; recovered
19F	3 doses	−	1:40	Severe symptoms; died 48 hours
20F	3 doses	−	1:80	Severe symptoms; recovered
21F	3 doses	−	1:40	Severe symptoms; recovered
22F	3 doses	−	1:10	Severe symptoms; died 72 hours
Control	0	−	0	Severe symptoms; died 24 hours
Control	0	−	0	Severe symptoms; died 24 hours
Control	0	−	0	Severe symptoms; recovered
Control	0	−	0	Severe symptoms; died 48 hours

* 100,000,000 per kilogram subcutaneously every 5 days.

† No agglutination in final dilution of 1:10.

‡ With 1 c.c. of suspension of 36-hour blood agar culture in saline solution.

to 48 hours while the remaining animal finally recovered after developing severe symptoms of meningitis.

Of four receiving five doses of the vaccine, three survived intracisternal inoculation and of four given three doses two survived.

All of the eight animals given the Type III vaccine developed varying amounts of agglutinin (table 5). Of four unvaccinated controls inoculated intracisternally, three succumbed in 24 to 48 hours while one recovered after developing severe symptoms.

Of the four given five doses of vaccine, three recovered after developing slight symptoms of meningitis while none of the four given three doses survived.

As shown in table 6, all twelve animals given three to five doses of the mixed vaccine of Types I and III developed varying amounts of agglutinins.

Of six inoculated intracisternally with Type I meningococcus, five recovered after developing symptoms of meningitis, but none of the six inoculated with Type III survived.

TABLE 5

RESULTS OF VACCINATION OF RABBITS WITH TYPE III MENINGOCOCCUS

RABBIT NUMBER	VACCINE	AGGLUTINATION REACTION		RESULTS OF INTRACISTERNAL INOCULATION‡
		Before vaccination	After vaccination	
1W	5 doses*	—†	1:10	Slight symptoms; recovered
2W	5 doses	—	1:160	Very slight symptoms; recovered
3W	5 doses	—	1:80	Severe symptoms; died 6th day
4W	5 doses	—	1:20	Slight symptoms; recovered
15W	3 doses	—	1:80	Severe symptoms; died 48 hours
16W	3 doses	—	1:40	Severe symptoms; died 72 hours
17W	3 doses	—	1:20	Severe symptoms; died 48 hours
18W	3 doses	—	1:40	Severe symptoms; died 48 hours
Control	0	—	0	Severe symptoms; died 36 hours
Control	0	—	0	Severe symptoms; died 24 hours
Control	0	—	0	Severe symptoms; recovered
Control	0	—	0	Severe symptoms; died 24 hours

* 100,000,000 per kilogram subcutaneously every 5 days.

† No agglutination in final dilutions of 1:10.

‡ With 1 c.c. of suspension of 36-hour blood agar culture in saline solution.

It will be noted, therefore, that the results observed with rabbits have been better than with guinea pigs, probably due in part at least to the fact that both types upon intracisternal inoculation were less virulent in the amounts given since two out of twelve unvaccinated controls recovered.

With Monkeys. As previously stated, neither strain in dose of 2 c.c. of suspension by intraspinal injection produced fatal meningitis in our control monkeys, although both produced

moderate signs and symptoms of meningitis, starting in about six hours after inoculation, reaching the maximum in about 24 hours with cloudy spinal fluids showing numerous meningococci in direct smears and cultures, but finally resulting in recovery. These symptoms were manifested by the fact that the animals sat on the floors of the cages with their heads down, or lying on

TABLE 6

RESULTS OF VACCINATION OF RABBITS WITH A MIXED VACCINE OF EQUAL PARTS OF TYPES I AND III MENINGOCOCCUS

RABBIT NUMBER	VACCINE	AGGLUTINATION REACTIONS		RESULTS OF INTRACISTERNAL INOCULATION‡	
		Before vaccination	After vaccination	With Type I	With Type III
9M	5 doses*	—†	1:160		Died 48 hours
10M	5 doses	—	1:160		Died 48 hours
11M	5 doses	—	1:40		Died 6 days
12M	5 doses	—	1:40	Recovered	
13M	5 doses	—	1:160	Recovered	
14M	5 doses	—	1:20	Recovered	
23M	3 doses	—	1:20		Died 24 hours
24M	3 doses	—	1:160		Died 48 hours
25M	3 doses	—	1:80		Died 24 hours
26M	3 doses	—	1:40	Died 5 days	
27M	3 doses	—	1:20	Recovered	
28M	3 doses	—	1:40	Recovered	
Control	0	—	0		Died 24 hours
Control	0	—	0		Died 36 hours
Control	0	—	0	Died 24 hours	
Control	0	—	0	Died 72 hours	

* 100,000,000 per kilogram subcutaneously of equal parts of Types I and III meningococcus every 5 days.

† No agglutination in final dilutions of 1:10 with an antigen of equal parts of Types I and III meningococci.

‡ With 1 c.c. of suspension of 36-hour blood agar culture in saline solution.

their sides, moving slowly and very reluctantly upon being disturbed, refusing to climb to perches, refusing to eat, slightly spastic in both arms and legs upon examination, etc.

However, the failure of the intraspinal inoculations to produce fatal infections has made the interpretation of the effects of vaccination of these animals more difficult than experienced with guinea pigs and rabbits.

As shown in Tables 7, 8 and 9, the subcutaneous injection of 4 or 5 doses of both vaccines resulted in the production of agglutinins and complement fixing antibodies, but the amounts of agglutinins produced were in most instances less than observed in the guinea pigs and rabbits.

TABLE 7

RESULTS OF VACCINATION OF MONKEYS WITH TYPE I MENINGOCOCCUS

NUMBER	WEIGHT	VACCINE	AGGLUTINATION	COMPLEMENT FIXATION	RESULTS OF INTRASPINAL INOCULATION WITH TYPE I*
	gms.				
3	2,700	5 doses†	1:40	4 4 3− −‡	No symptoms
4	4,700	5 doses	1:10	3 2 1− −	Hind legs; recovered
7	3,200	5 doses	1:20	4 1− − −	No symptoms
9	2,800	4 doses	1:40	4 4 3− −	No symptoms
10	3,000	4 doses	1:30	4 2 2 1−	No symptoms
Control	2,700	0	Neg. 1:10	2 1− − −	Moderate symptoms; recovered
Control	3,000	0	Neg. 1:10	1− − − −	Moderate symptoms; recovered

* With 2 c.c. suspension of 36-hour blood agar culture in saline solution.

† 100,000,000 per kilogram subcutaneously every 5 days.

‡ Conducted with following amounts of serum: 0.05, 0.025, 0.0125, 0.006 and 0.003 c.c.; 4 = ++++; 3 = +++, etc.

TABLE 8

RESULTS OF VACCINATION OF MONKEYS WITH TYPE III MENINGOCOCCUS

NUMBER	WEIGHT	VACCINE	AGGLUTINATION	COMPLEMENT FIXATION	RESULTS OF INTRASPINAL INOCULATION WITH TYPE III*
	gms.				
1	4,500	5 doses†	1:20	4 4 4− −‡	No symptoms
2	4,000	5 doses	1:10	4 2− − −	No symptoms
3	2,700	5 doses	1:20	3 1− − −	Hind legs; recovered
7	3,600	4 doses	1:40	4 4 4 4−	Hind legs; recovered
8	2,100	4 doses	1:40	4 4 4 4−	No symptoms
Control	3,000	0	Neg. 1:10	2 1− − −	Moderate symptoms; recovered
Control	3,400	0	Neg. 1:10	1− − − −	Moderate symptoms; recovered

* With 2 c.c. suspension of 36-hour blood agar culture in saline solution.

† 100,000,000 per kilogram subcutaneously every 5 days.

‡ Conducted with following amounts of serum: 0.05, 0.025, 0.0125, 0.06 and 0.003 c.c.; 4 = −−−−; 3 = −−−, etc.

If, however, it is permissible to draw deductions on the basis of the severity of signs and symptoms among the vaccinated animals as compared with the unvaccinated controls, it would appear that the administration of 4 and 5 doses of the vaccine of Type I (table 7) and Type III (table 8) as well as of a mixture of these two vaccines (table 9), had some immunizing effect since, of the 13 vaccinated animals, 10 remained perfectly well following recovery from the anesthetic, while 3 showed some weakness and spasticity of the hind legs. Since all of these

TABLE 9

RESULTS OF VACCINATION OF MONKEYS WITH A MIXED VACCINE OF TYPES I AND III MENINGOCOCCUS

NUMBER	WEIGHT	VACCINE	AGGLUTINATION	COMPLEMENT FIXATION*	RESULTS OF INTRASPINAL INOCULATION†	
					With Type I	With Type III
	gms.					
6	2,800	5 doses‡	1:30	4 4 4 2−§	No symptoms	
11	3,800	4 doses	1:20	4 4 3 1−		No symptoms
12	4,000	4 doses	1:40	4 4 4 1−		No symptoms
Control	3,200	0	Neg. 1:10	2− − − −	Marked symptoms; recovered	
Control	2,800	0	Neg. 1:10	2 1− − −		Marked symptoms; recovered

* With homologous strains.

† With 2 c.c. of suspension of 36-hour blood agar culture in saline solution.

‡ 100,000,000 per kilogram subcutaneously of equal parts of Types I and III meningococcus every 5 days.

§ Conducted with following amounts of serum: 0.05, 0.025, 0.0125, 0.006 and 0.003 c.c.; 4 = ++++; 3 = +++, etc.

animals continued to eat and appeared otherwise well, as well as showing perfectly clear spinal fluids 24 hours after intraspinal inoculation, it was a question whether or not the involvement of the hind legs was actually due to meningitis or the result of trauma incident to the intraspinal inoculations. All of the six unvaccinated controls showed moderate symptoms of meningitis about 24 hours after inoculation, with cloudy spinal fluids. At the end of 72 hours, however, the fluids had become perfectly clear with disappearance of all symptoms and complete recovery.

DISCUSSION

As shown in table 10, the general results of this investigation have indicated that some degree of immunity against virulent meningococci may be engendered among guinea pigs, rabbits and monkeys by active immunization and lends encouragement to further efforts toward the vaccination of human beings against meningococcus meningitis. Certainly the kind of vaccine and doses administered have resulted in the production of agglutinins and complement-fixing antibody (monkeys) and while the percentage of immunized guinea pigs and rabbits surviving intra-

TABLE 10

SUMMARY OF RESULTS OF VACCINATION AGAINST EXPERIMENTAL MENINGOCOCCUS MENINGITIS

ANIMAL	NUMBER VACCINATED	NUMBER SURVIVING INOCULATION	PERCENTAGE SURVIVING OR REMAINING WELL
			per cent
Guinea pigs	28	5	18
	12 controls	None	0
Rabbits	28	13	46.4
	12 controls	2	16.6
Monkeys	13	Three symptoms; all survived	77
	6 controls	All symptoms; all survived	0

cisternal inoculations of virulent organisms has been small, yet this test for acquired immunity is admittedly severe. While our animals were tested for acquired immunity, about four weeks after the last dose of vaccine, it may be that a longer interval may have shown a higher degree of immunity, as indicated by the results of skin tests by Kuhns among men following vaccination with meningococcus broth filtrates.

Since the attack rate of meningococcus meningitis is usually low, mass immunization has received but little encouragement and certainly the evidence in favor of such vaccination as a protective measure is by no means conclusive at the present time. But if positive intracutaneous reactions to exotoxin or whole

broth culture sterilized with merthiolate or other chemical agents (Ferry and Steele; Kuhns) are ultimately proven a reliable index of susceptibility to infection along with the converse, that negative skin reactions due to natural immunity and vaccination are indicative of effectual resistance, encouragement would be given to the vaccination of susceptible individuals. Using a mixture of the filtrates of broth cultures of Types I and III sterilized with 1:10000 merthiolate as a vaccine, Kuhns has observed that 80 men giving positive skin reactions before the administration of four doses of vaccine, 6.9 per cent gave negative skin reactions when tested six weeks after the last dose and when 41 were retested four months later, 72 per cent gave negative reactions. Skin tests conducted with whole broth cultures sterilized with merthiolate gave more pronounced reactions than tests with filtrates (exotoxins) alone and, in our opinion, it is likely that vaccines incorporating the exotoxins as well as the chemically killed cocci themselves, such as used in this investigation, will prove more effective than exotoxins alone since it is hardly likely that natural and acquired immunity to the meningococcus are purely antitoxic in nature. This, however, awaits the results of further investigation. While we have not included in this study the vaccination of the lower animals with broth filtrates alone, it is likely that these are capable of engendering some degree of effective resistance as indicated by the report of Ferry[19], who observed that of six monkeys inoculated intracisternally, two to three months previously with toxin or toxin-antitoxin mixtures, four survived intraspinal inoculation with a known fatal dose of living meningococci.

SUMMARY

Guinea pigs, rabbits and monkeys have been immunized by the subcutaneous injection of 3 to 5 doses at weekly intervals of vaccines of Types I and III meningococci cultivated in hormone broth for five days and sterilized with tricresol.

All have shown the production of varying amounts of agglutinin. The sera of immunized monkeys also showed the presence of complement fixing antibody.

Four weeks after the last doses of the vaccines the guinea pigs and rabbits were tested for acquired resistance by the intracisternal inoculation of living virulent meningococci. The monkeys were tested by the intraspinal inoculation of the organisms.

All, or 100 per cent, of 12 unvaccinated control guinea pigs succumbed to severe meningitis in 24 to 72 hours. Of 28 vaccinated animals 5, or 18 per cent, recovered.

Of 12 unvaccinated control rabbits 10, or 83.4 per cent, succumbed. Of 28 immunized animals 15, or 53.6 per cent, succumbed and 13, or 46.4 per cent, recovered.

All of 6 unvaccinated control monkeys showed moderately severe symptoms of meningitis, but recovered. Of 13 immunized animals, 3 probably showed symptoms of meningitis with recovery, while the remaining 10, or 77 per cent, remained entirely well.

These results are believed to lend encouragement to efforts for the vaccination of human beings against meningococcus meningitis and especially if skin or other tests are found reliable for the detection of susceptible individuals.

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STREPTOCOCCAL VACCINES IN THE PREVENTION AND TREATMENT OF RESPIRATORY INFECTIONS

A Clinical and Experimental Study*

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Since immunity following epidemic respiratory infections, including influenza, is of short duration, any preventive agent or vaccine must be harmless, generally and repeatedly applicable, and readily obtainable in large amounts. It is becoming increasingly apparent that whatever the causative rôle of the viruses of the common cold and influenza may be, the cultivable organisms, chiefly streptococci or pneumococci, are the main cause of the symptoms, lesions and deaths. It has been conclusively shown that immunity of short duration can be produced in humans and animals with vaccines prepared from heat-killed streptococci and pneumococci[1, 2, 3]. A diminished incidence of influenza and its complications was produced with a vaccine containing freshly isolated strains of streptococci and pneumococci on a nation-wide scale during the pandemic of influenza of 1918–1920, when the streptococcus generally present had pandemic characteristics[5, 7]. Objectionable local and constitutional reactions sometimes occurred following the injection of the vaccine. In a later study, made in more normal times[6], preventive and curative results were again obtained. The vaccine used in the second study was prepared, not directly from cultures as in the previous study, but from streptococci preserved in dense suspension of glycerin (2 parts) and 25 per cent physiologic saline solution (1 part). It was found that heat-killed vaccines prepared from dilutions of the dense suspensions were much less toxic and more highly antigenic than those prepared

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directly from cultures and caused a more rapid, and more favorable, response in human beings and mice.

It was found also that the distribution curves of cataphoretic velocity of the streptococci at hand in epidemics of the common cold and of influenza, while very different, were characteristic in widely separated outbreaks, both as to time and place. By means of cataphoresis and experiments in mice it was found that serologic immunity and protection occurred cross-wise following injection of streptococci obtained in cases of colds and of influenza, respectively, and that vaccines prepared with streptococci obtained in cases of influenza had wider protective effects.

On the basis of these findings large amounts of the respective freshly isolated streptococci were placed in the glycerin-salt solution menstruum in the hope that suitable preventive and curative vaccines might be thus available in advance of, or abreast with, subsequent outbreaks of epidemic respiratory infections.

Through the kind coöperation of physicians in various parts of the United States and Canada, and of physicians of The Mayo Clinic and of The Mayo Foundation, we were able to make a further study of this inherently difficult and important problem. New methods for producing and determining immunity in animals, and for determining characteristic virulence of the streptococci obtained in cases of colds and influenza have been discovered. It is the purpose of this paper to report on these further studies.

METHODS

The streptococci incorporated in the vaccines used in this study were isolated from the nasopharynx or sputum in cases of the common cold and influenza, respectively. The primary isolations were made in dextrose-brain broth. If the cataphoretic velocity was typical, or if the strain caused hemorrhagic edema of the lungs or bronchopneumonia in rabbits following intracerebral inoculation, and if platings revealed a pure culture of the streptococci, subcultures were made in dextrose-brain broth in rapid succession three to five times. Large volumes of previously warmed, 0.2 per cent dextrose broth were then inoculated. Each bottle containing 3,500 c.c. received the entire young culture in one tube of dextrose-brain broth, or approximately 20 c.c. As soon as abundant growth had occurred the organisms were harvested in a continuous

feed centrifuge and the putty-like mass suspended in glycerin (2 parts) and 25 per cent solution of sodium chloride (1 part). Glass beads were added and the mixture emulsified by shaking in a shaking machine. The vaccines were made as needed, preferably not before one year after preservation, by diluting the dense suspension with sodium chloride solution to about the density of a broth culture (2,000,000,000 organisms per cubic centimeter) and heating to 75°C. for one hour. Phenol to make 0.2 per cent was added as a preservative.

For several years the "cold" streptococcal vaccine was given for prophylaxis in autumn as colds began to appear, and then once a month up to January. The "influenza" streptococcal vaccine was then given for the remainder of the winter and in the spring. For several years we observed that influenzal types of respiratory infections began increasingly earlier in the autumn. This was particularly true last year. Hence, during the past year (1936) a mixture of equal parts of the two types of vaccine was mostly used throughout the prevalence of respiratory infections. Routinely, 0.3, 0.5 and 1.0 c.c. were given subcutaneously a week apart, and then 1.0 c.c. once every three or four weeks. It was advised that the dosage be reduced in case of untoward reaction. Those who took the prophylactic injections were advised to return immediately for treatment should symptoms of a cold or of influenza develop. For treatment, the initial dosages, or smaller amounts at intervals of forty-eight hours, depending on results obtained, were recommended.

Impressed by the work of Ross[3, 9] and Powell, and Rockwell and van Kirk[4] on the results from vaccination by mouth, we prepared a vaccine for this purpose by adding one part of a saline suspension, of suitable density, of the heat-killed streptococci (to which phenol was added to make 0.2 per cent) from the dense suspension in glycerin-salt solution, to four parts of a concentrated solution of dextrose, maltose, dextrins and saccharose in the form of Karo syrup, the final concentration being 20,000,000,000 organisms per cubic centimeter. This vaccine is flavored with oil of peppermint and colored pink with a harmless dye. Vaccines prepared by this method have a number of advantages. Dosage can readily be changed for particular needs. Owing to the high concentration of hygroscopic sugars the bacteria are dehydrated, contaminants cannot grow because of the absence of free water, and hence refrigeration is not necessary. The organisms should retain their specific antigenicity indefinitely. We have been using this vaccine for the prevention of respiratory infections and in treatment with seemingly striking results. Five drops are taken directly on the tongue or are allowed to trickle into the pharynx through the nostrils while the head is held in a retracted position, or the vaccine may be taken in water, preferably a half to one hour before breakfast. For prophylaxis the dosage is increased by five drops daily, up to twenty drops; then twenty drops are to be taken once or twice weekly, provided no untoward symptoms develop. Similar dosages are used for treatment. The results from its use in human beings are not included in the tables.

CLINICAL RESULTS

The results in prophylaxis obtained by us and by physicians to whom the vaccine was sent for study are summarized in table 1. The persons vaccinated were chiefly those who were, for one reason or another, abnormally susceptible to recurring respiratory infections. It will be noted that in the great majority of the different groups representative of different climatic conditions, the incidence of colds and of influenza was greatly reduced. A lesser number were slightly benefited and very few were not benefited. The results obtained for vaccinated and unvaccinated

TABLE 1

RESULTS FROM PROPHYLACTIC USE OF "COLD AND INFLUENZA" STREPTOCOCCAL VACCINES

GROUPS	PERSONS VACCINATED	INCIDENCE OF									
		Colds					Influenza				
		Reduced			Not reduced	Increased	Reduced			Not reduced	Increased
		Markedly	Slightly	Per cent			Markedly	Slightly	Per cent		
Rochester, Minnesota	57	32	20	91	5	0	28	25	93	4	0
Atlantic states	610	383	134	92	40	5	100	11	95	5	1
Southern states	671	432	109	95	29	0	143	51	85	35	
Central states	4,145	3,089	655	93	261	30	1,153	167	94	85	1
Western states	1,410	731	380	86	184		153	37	83	36	1
Total	6,893	4,667	1,298	91	519	35	1,577	291	91	165	3

persons living under comparable conditions and with undue exposure, are summarized in table 2. The three groups of nurses were from widely separated hospitals. There were eleven separate camps of the Civilian Conservation Corps, all in Minnesota, in each of which some of the men were vaccinated and some were not. Changes in the population among the vaccinated and unvaccinated were about the same and hence the figures are fairly comparable. In three other camps all men had been vaccinated, and in ten additional camps, also in Minnesota, none was vaccinated. It will be seen that the incidence of colds or

influenza was consistently from a third to a half as great among the vaccinated as among the unvaccinated. In each of the fourteen sub-groups, comprising the two first groups in the table, of which some were and some were not vaccinated, the vacci-

TABLE 2

RESULTS IN VACCINATED AND UNVACCINATED PERSONS LIVING UNDER COMPARABLE CONDITIONS

GROUPS	NUMBER OF GROUPS	NUMBER OF PERSONS	INCIDENCE OF CASES OF:			
			Colds or influenza		Pneumonia	
			Number	Per cent	Number	Per cent
Nurses:						
Vaccinated	3	87	22	25	0	0
Unvaccinated		107	44	41	0	0
Registrants, CCC camps:						
Vaccinated	11	644	75	12	1	0.15
Unvaccinated		1,440	488	34	3	0.21
Registrants, CCC camps:						
Vaccinated	3	596	88	15	0	0
Unvaccinated	10	1,465	420	29	3	0.20

TABLE 3

RESULTS FROM USE OF "COLD AND INFLUENZA" STREPTOCOCCAL VACCINES IN TREATMENT

DISEASES	PATIENTS TREATED	BENEFITED			NOT BENEFITED	MADE WORSE
		Markedly	Slightly	Per cent		
Colds	3,083	1,924	746	87	344	69
Influenza	1,412	1,006	275	91	127	4
Pneumonia	131	95	25	91	11	0
Chronic bronchitis	272	189	55	90	28	0
Chronic sinusitis	526	342	130	90	53	1

nated fared better as regards incidence, severity and duration of respiratory infections than did the unvaccinated controls.

In table 3 are given the results from the use of the vaccine in treatment. From each of the climatic groups good effects were

reported in a high percentage of cases of both acute and chronic respiratory infections. The incidence of so-called complications among 6,712 persons adequately vaccinated was as follows: sinusitis, twenty-one; otitis media, seven; mastoiditis, none; pneumonia, two; empyema, none; encephalitis, none; deaths from pneumonia, none. This incidence was clearly much lower than that of an estimated equal number of unvaccinated persons living in the same communities, but the figures pertaining to the latter groups were too indefinite to permit making close comparisons. The vaccine was used by some in the treatment of systemic diseases which commonly follow acute respiratory infections. Favorable results were reported in twenty-four cases of bronchial asthma, in several cases of multiple sclerosis and of chronic encephalitis, and in a number of cases each of acute otitis media, mastoiditis and sinusitis. Nearly all of the 214 physicians who reported by questionnaire had favorable results, and all but four of the sixty-five who reported by letter were favorably impressed with the results from the use of the vaccine, either as a prophylactic or therapeutic measure, or both.

IMMUNIZATION OF MICE AND RATS

The "cold and influenza" streptococcal vaccines used for human beings were employed in dilutions of 1 to 10 for the immunization of mice. Increasing doses of from 0.1 to 1.0 c.c. of the saline vaccine were injected subcutaneously on alternate days for ten days. The oral "cold and influenza" streptococcal vaccine, also diluted 1 to 10, was used to immunize mice and rats by nasal aspiration, by dropping it into the nose and introducing it into the stomach (2 c.c.) through a ureteral catheter during ether anesthesia (table 4) on alternate days for ten days, and by supplying it and a similarly prepared Type I pneumococcus vaccine instead of water for drinking (table 5). It was estimated that each mouse drank approximately 7.5 c.c. and each rat approximately 20 c.c., of the diluted vaccine per day. Immunization was continued for seven to ten days before test inoculations and thereafter.

During a study of the recent epidemic of influenza a new method was found for testing pneumotropic virulence of the streptococci isolated. This consisted simply of having mice and rats, while under ether anesthesia, aspirate cultures of freshly isolated strains of streptococci into the lungs by immersing their noses in the culture. We have used this method, which simulates the route by which respiratory infections are considered to occur under natural conditions, to compare the resistance of vaccinated and unvaccinated mice and rats.

Eight different strains of streptococci isolated from the nasopharynx, blood, sputum or lungs of patients who had influenza in the 1937 epidemic, and one strain of Type I pneumococcus, were used in the different experiments summarized in tables 4 and 5. They had been subcultured in rapid succession from four to sixteen times in dextrose-brain broth or lung-mash medium and had been passed through one to four animals, then preserved without transfer for from a half month to three months in chick-mash or lung-mash mediums layered with oil. They had lost much of the high virulence they possessed on isolation but had retained pneumotropic virulence suitable for these comparative tests. The lesions of the lungs of the animals that died following aspiration of strepto-

TABLE 4

IMMUNIZATION OF MICE AGAINST STREPTOCOCCI FROM INFLUENZA

MICE IMMUNIZED WITH:	METHOD OF IMMUNIZATION	NUMBER OF:		PERCENTAGE WITH LESIONS OF LUNGS	PERCENTAGE INCIDENCE OF ISOLATION OF STREPTOCOCCI FROM:			
		Experiments	Mice		Lungs	Spleen	Liver	Brain
Saline vaccine (1:10)	Subcutaneous	4	24	13	13	18	13	8
Oral vaccine in syrup (1:10)	Nasal aspiration	3	18	6	0	0	6	0
Oral vaccine in syrup (1:10)	Nostrils + stomach	4	24	7	4	0	0	0
Total			66	9	6	11	11	3
Not immunized	0	4	24	92	71	54	50	13
Saline solution	Subcutaneous	2	10	100	60	50	50	40
Saline solution	Nasal aspiration	2	11	45	45	36	9	9
Syrup (1:10)	Nasal aspiration	2	10	60	60	40	30	20
Total			55	78	62	47	38	20

cocci obtained in cases of influenza consisted chiefly of acute hemorrhagic edema with interspersed regions of emphysema and bronchopneumonia; lesions were of diffuse hemorrhagic or gray consolidation, lobar in distribution, following aspiration of Type I pneumococci. The animals that did not succumb were etherized for examination three to six days following inoculation. Conditions as to inoculation, feeding, examination and culture of the immunized and control animals were made as comparable as possible. Cultures from pieces of the organs, approximately of equal size, in the test and control groups, were made in dextrose-brain broth. Smears were made twenty-four to forty-eight hours later from all tubes that showed growth and the type of organism found was recorded.

The results from the immunization of mice and rats with these vaccines, as shown by mortality rate, incidence of lesions of lungs, and of isolation of streptococci from the different organs, are summarized in tables 4 and 5. It will

TABLE 5

ORAL IMMUNIZATION OF MICE AND RATS AGAINST TYPE I PNEUMOCOCCI AND STREPTOCOCCI FROM COLDS AND INFLUENZA

TEST ANIMALS	IMMUNIZING ANTIGENS		TEST STRAINS	NUMBER OF ANIMALS	MORTALITY	LESIONS OF LUNGS	PERCENTAGE INCIDENCE OF ISOLATION OF STREPTOCOCCI FROM:				
							Lungs	Spleen	Liver	Brain	Heart
					per cent	*per cent*					
Mice	Vaccines prepared with streptococci from colds and influenza after glycerolation for:	¼ to 5 years (#17067)	A*	10	0	10	10	0	0	0	0
		17 to 19 years (#1404)	A	10	20	30	30	20	10	10	10
		Control	A	14	36	79	57	87	75	71	29
Rats		¼ to 5 years (#17067)	A	9	0	0	0	0	11	0	11
		Control	A	9	11	100	67	44	56	44	22
		½ to 5 years (#16420)	B†	9	0	0	0	0	0	0	0
		Control	B	8	25	100	13	25	38	38	38
	Type I pneumococci after glycerolation for 15 years		C‡	9	0	0	0	0	0	11	0
	Control		C	9	11	44	67	22	33	22	22

* A = mixture of 3 strains from influenza, 1937.
† B = mixture of 2 strains from influenza, 1937.
‡ C = Type I pneumococcus from pneumonia, 1937.

be noted that a high degree of resistance to the streptococcus obtained in cases of influenza was induced with the streptococcal vaccines by each of the several methods of vaccination, and also to Type I pneumococcus with Type I pneumococcus vaccine given by the oral route.

COMMENT AND SUMMARY

We realize how difficult it is to evaluate accurately the degree of protection and the therapeutic effects of vaccines generally. The clinical results obtained everywhere, however, including comparable groups of vaccinated and unvaccinated persons, and the results obtained experimentally in mice and rats under controlled conditions, were so favorable with our vaccines as to leave no reasonable doubt of their general efficacy and immunizing action in prophylaxis and treatment of the usual epidemic and other respiratory infections. The high protective and curative actions we believe were due to the methods used in the preparation and use of the vaccine. Most important was the use of many freshly isolated strains the antigenicity of which was preserved and the toxicity of which was reduced during prolonged preservation in the glycerin-salt solution menstruum.

In accord with the idea that streptococci are the cause of symptoms is the fact that symptoms resembling those of a cold or influenza may be precipitated or aggravated at the beginning of attacks in highly sensitive individuals by administering too large a dose of vaccine. For a number of years in the spring months when mild upper respiratory infections were prevalent we have found an unmistakable lack of the usual antibody-like response to the vaccine when it was given as treatment during the early stages of respiratory infections, both to vaccinated and to unvaccinated persons. This we interpreted as owing to a change in the streptococcal flora of respiratory infections in spring, notwithstanding the fact that the cataphoretic velocity of the streptococci was found similar to that of the streptococci in the vaccine. We have learned since that persons, both vaccinated and unvaccinated, are prone, for reasons yet obscure[10], to be more sensitive in the spring as compared with other seasons, to injection of vaccines, and that lack of response was owing not to a "misfit" of the vaccine but to relative overdosage. By giving oftener approximately half the routine dosage, the usual favorable results have since been obtained.

The vaccine prepared at present from a mixture of 400 strains of streptococci isolated in the pandemic of influenza of 1918–1920, which have been preserved in dense suspension of glycerin-salt solution ever since, afforded protection to mice, against streptococci isolated in the epidemic of 1937 (table 5). Likewise, a vaccine prepared from a strain of Type I pneumococcus, similarly preserved for fifteen years, protected rats and mice against a strain recently isolated in a case of lobar pneumonia.

The streptococcal-pneumococcal flora of current epidemic and sporadic respiratory infections, as shown by cataphoresis, by experiments on animals[6], and by this study, is antigenically not as heterogenous as is usually assumed, but sufficiently homogenous that composite vaccines such as we have used should possess wide applicability.

Methods for the preparation and use of relatively nontoxic, highly antigenic vaccines which are suitable for prevention and treatment of respiratory infections, and which can be available in advance of epidemic outbreaks, are reported.

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PERIPELVIC LYMPHATIC CYSTS OF THE KIDNEY

A Review of the Literature on Perinephric Cysts*

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For many years it has been recognized that cysts occur in the lymphatics of the hilus of the kidney and it is apparent that a number of pathologists have observed cysts of this type. Wildbolz[34] and Klebs[16], for instance, have listed lymphatic cysts as one of several types of pararenal cysts. Berner[7] suggested dilated lymphatics as a possible, although improbable, cause of cysts in the substance of the kidney in two cases in his series. There is, however, a dearth of accurate descriptions of lymphatic cysts in the region of the hilus of the kidney, presumably because such lesions very rarely, if ever, attain sufficient size to become clinically important.

The first and most complete description of peripelvic lymphatic cysts was given by Rivalta[29], who, in 1889, reported the lesion as an incidental finding at necropsy on two elderly patients observed under the direction of von Recklinghausen. Rivalta recognized that the cysts were lymphatic ectasias and quoted Virchow[33] as authority for the occurrence of an analogous lesion in the human being; namely, dilated lymphatics of the female pelvis following puerperal inflammatory disease.

Perirenal cysts are apparently uncommon among lower animals, but Schmey[31] reported observations on three swine in which he found perirenal cysts or "cystoids." Schmey believed that these cysts probably were the result of lymph stasis. Minkowski[20] found a large perinephric cyst in a man aged twenty-one years, who also had polycythemia. Minkowski apparently did

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not believe that this cyst was a lymphatic cyst but said that Hoffmann[13] observed dilatation of the renal lymphatics after obstruction of the hilus of the kidney by carcinoma. One other case, reported by Malherbe[19], is important in a consideration of the possible relationship of some of the large perinephric cysts and lymphatic cysts. At the time the patient was observed by Malherbe, he had a large perinephric cyst which was treated by nephrectomy. The possibility that the cyst in this instance was caused by lymphatic stasis is now suggested by a previous history of renal colic and hematuria in this case, and the fact that Malherbe described a number of small lacunae in the fat at the hilus of the kidney. Lymphatic cysts in our series have often had a similar appearance.

Other cases of perinephric cysts have been reported. Albarran and Imbert[5] collected seventeen examples from the literature. In several instances (Baldwin[6], Lockwood[18], Abbe[1], Prudden[27], Newman[22], and Périer[26]), cysts have been reported without discussion of the pathogenesis by the author. Several authors, Pawlik[25], Hawkins[10], Kaiser[15], and Damm[8], reported cases in which a cyst was traumatic in origin.

Another group of authors, Obalinski[23], Przewoski[28], and Albarran[4], have presented the theory that perinephric cysts arise from embryonic remnants of the wolffian body. Indeed, in the case reported by Albarran, the patient was a child two months of age.

Lecène and Thévenot[17] have given general discussions on the subject of perinephric cysts and suggested encysted hematoma, lymphatic cysts, and cystic neoplasms as etiologic factors. These authors called attention to the distinction between cysts communicating with the renal pelvis and those entirely distinct from the pelvis. Hepler[11], and Hinman and Hepler[12], apparently have furnished a brilliant solution to the question of the pathogenesis of cysts which communicate with the renal pelvis. They produced cysts of this type experimentally by combining infarction of a portion of the kidney with hydronephrosis. In cases reported by Morris[21], Adler[3], and Haslinger[9], the cysts communicated with the renal pelvis.

The references cited here do not exhaust the literature on perinephric cysts, but the representative reports which have been listed indicate that there are probably several different causes of thin-walled cysts with serous content outside of the renal substance. Dermoid cysts, cystic neoplasms, echinococcus cysts, and so forth, are not considered here because their etiology and characteristics are clearly different from lymphatic cysts.

Recently, attention has been called to the possible lymphangiomatous source of certain cysts observed in the region of the kidney hilus. Such cysts were found in fifteen (1.28 per cent) of a series of 1,175 necropsies. Later, five more of these cysts were found in another series. The age and sex of the patients and the nature of the associated lesions in these cases were analyzed in table 1 from which it is immediately apparent that the disease is predominant among males for only four of the patients were women.

The age incidence varied from seventeen to seventy-one years, but only three patients were less than fifty years of age and the average age incidence was sixty and a half years. The presence of scars and cortical cysts in most of the kidneys suggests an inflammatory basis for the peripelvic cysts. It is striking in this regard that the youngest patient in this series (case 13) gave a definite history of nephritis and edema.

In eleven of the other cases in which the patients were men, there was a history or evidence of obstructive, infectious, or calculus disease of the kidney.

One other patient (case 4) had been subjected to an operation on the sigmoid colon many years previously, which conceivably may have produced temporary urinary obstruction or infection. Three of the four women had borne children and may have had renal infection during the puerperium. It is also rather striking that in eight cases the patients had lesions that generally are presumed to be congenital anomalies; lymphatic ectasia occurred in other organs in five of these cases. From this fact, it is evident that on a statistical basis alone, the evidence for the congenital origin of peripelvic lymphatic cysts is just as good as the evidence for the inflammatory origin. The fact that the cysts occurred late in life, however, is in favor of an acquired lesion.

TABLE 1

CLINICAL DATA IN CASES OF PERIPELVIC LYMPHATIC CYSTS OF THE KIDNEY

CASE	PATIENTS		CORTICAL CYSTS OF KIDNEYS, GRADE*	SCARS OF KIDNEYS, GRADE*	CONGENITAL ANOMALIES	PREDISPOSITION TO RENAL INFLAMMATION
	Age	Sex				
	years					
1	66	M	1	2	None	Carcinoma of bladder
2	59	F	1	2	Suprarenal rests in kidneys	None; no pregnancy
3	70	M	1	1	Lymphangioma of spleen	Nephrolithiasis; hypertrophy of prostate gland, grade 1
4	68	M	1	1	None	Resection performed for carcinoma of sigmoid colon, twenty-four years before death
5	71	F	1	2	None	One child
6	74	F	2	1	Cysts of liver and pancreas; lymphangioma of spleen	Five children
7	52	M	1	1	Lymphangioma of jejunum	Median bar; hypertrophy of prostate gland, grade 1+
8	72	F	2	2	None	Two children; pelvic carcinoma
9	69	M	1	2	Patent foramen ovale	Hypertrophy of prostate gland, grade 2
10	66	M	0	1	None	Hypertrophy of prostate gland, grade 2+
11	45	M	2	0	None	None
12	65	M	2	1	None	Hypertrophy of prostate gland, grade 1; median bar
13	17	M	1	2	None	Recurrent albuminuria and edema
14	67	M	1	0	Hemangioma of liver; lymphangioma of spleen	Right nephrectomy performed, ten years before death; renal stones
15	64	M	1	2	Lymphangioma of suprarenal gland	Stone in right renal pelvis
16	70	M	1	2	Hemangioma of ileum	None
17	36	M	1	2	None	Stone in left renal pelvis
18	59	M	1	2	None	None
19	63	M	0	1	None	Hypertrophy of prostate gland, grade 1
20	56	M	0	0	None	None

* Graded on basis of 1 to 4.

Perinephric cysts were an incidental finding at necropsy in all cases, with the possible exception of case 5, in which there was a palpable right kidney and an intravenous urogram suggested the possibility of a small cyst.

The causes of death in these cases varied and obviously were unrelated to the peripelvic cysts.

FIG. 1. CYSTS IN PERIPELVIC FAT; OUTSIDE SURFACE OF KIDNEY IN CASE 1

In ten cases the peripelvic cysts were multiple and bilateral, multiple and unilateral in three cases, and a single cyst occurred in one renal hilus in seven cases. In case 14, right nephrectomy had been performed ten years before the death of the patient. The cysts had thin walls and contained a thin, clear, albuminous fluid that was apparently devoid of any kind of pigment.

The cysts varied in size from a microscopic lymphatic ectasia

to 5 cm. in diameter. A rough average diameter of the cysts, as seen by the naked eye, would be about 1 cm. When the cysts were multiple, they were distributed about the major blood vessels of the kidney on all sides, roughly in the position of the large lymphatic trunks which drain into the hilus from the

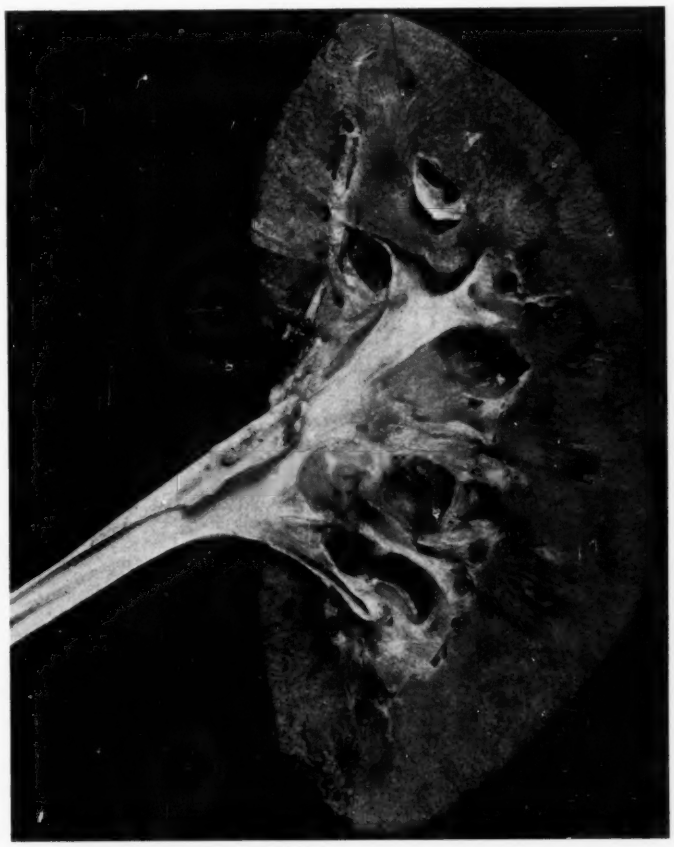

FIG. 2. CYSTS IN PERIPELVIC FAT; CUT SURFACE OF KIDNEY IN CASE 7

kidney and which have been described by such anatomists as Parker[24], Ssysganow[32], and Rouvière.[30] In spite of the fact that lymphatic drainage of the renal pelvis is sufficiently rich and that lymphatic injection has been observed in pyelograms by Abeshouse[2], Jarre[14] and others, connection between the cysts and

the renal pelvis was not demonstrated by any method, including injection of India ink and colored gelatin into some of the cysts. The gross appearance of the cysts is shown by figures 1 and 2, which respectively demonstrate the appearance of the outside surface of the kidney in case 1 and the cut surface of the kidney in case 7.

FIG. 3. LYMPHATIC CYSTS IN ANGIOMATOUS ARRANGEMENT

The epithelium of the renal pelvis is shown at the lower left portion; stained with hematoxylin and eosin (×80).

Microscopically, the cysts were lined with endothelium and supported by a thin wall of connective tissue. Figure 3 illustrates the lymphatic cysts in an angiomatous arrangement. In this section, there are a few mononuclear leukocytes, some of which contain hemosiderin. In seven cases in this series a

single section across the vessels of the hilus and peripelvic fat of the kidney sufficed to demonstrate hyaline thrombi of the lymphatic trunks proximal to the kidney. A probable basis for development of these thrombi is that of resolved inflammation.

Anatomically, the lymphatics of the kidney circulate in the parenchyma parallel with the renal blood vessels, and excellent

FIG. 4. DILATED LYMPHATIC OF SUBSTANCE OF KIDNEY; STAINED WITH HEMATOXYLIN AND EOSIN (×8)

evidence that the cysts are actually dilated lymph vessels is illustrated in figure 4, which is a low-power photomicrograph of a type of dilated lymph vessel of the renal substance seen in cases in which the cysts are large and multiple. A hyaline thrombus is present in this last figure at the point where the lymph vessel leaves the kidney. This particular section was taken from case 5.

Dilatation of the lymphatics of the renal parenchyma suggests the possibility that some of the cysts of the renal cortex are lymphatic in origin. This suggestion is given weight by the frequent association of rather large (up to 5 cm.) serous cortical cysts and lymphatic cysts of the hilus, but I have never observed thrombosis of the lymphatics of the renal substance in the region of cortical serous cysts.

In view of the histologic evidence just presented, it seems unlikely that epithelial remnants from the wolffian body or other embryologic structures are responsible for formation of the type of cyst herein described, because the cysts in all instances are lined by flattened endotheloid cells and are apparently related to dilated perivascular lymphatics in the renal substance and to dilated lymphatics of nerves. An obscure embryologic peculiarity in the individual cases, however, has not been ruled out. For example, it has not been determined why collateral circulation of lymph through the renal capsule does not carry away the collected lymph in the event of obstruction of the lymphatics of the hilus. Problems of lymph pressure and osmotic fluid balance in relation to lymph cyst formation cannot be settled from the anatomic standpoint alone.

It is evident then that, although there is good anatomic evidence that the cysts described here are lymphatic cysts, there is no evidence that satisfactorily explains the pathologic physiology involved.

SUMMARY

A type of perinephric cyst, which is evidently a lymphatic ectasia associated with thrombosis of the lymphatic trunks of the hilus of the kidney, has been described. The cysts attained clinical importance in size in possibly one case of twenty observed. It is my present opinion that cortical cysts of the kidney are not related to dilated lymphatics.

An article by Kretschmer, H. L., and Hibbs, W. G.: Retroperitoneal perirenal lymphangioma. Arch. Surg. **29**: 113–125 (July), 1934, should be added to the bibliography. The lymphangioma reported by these authors is apparently a true tumor but their viewpoint and bibliography are important to students of perinephric cysts.

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THE WASSERMANN REACTION IN INFECTIOUS MONONUCLEOSIS

With Report of a Case with Unusual Clinical Features*

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The interesting clinical manifestations of infectious mononucleosis and glandular fever have been rather completely described in the literature and Tidy, in a Lumelian lecture[1] has published an excellent review.

More recently, interest has been focussed on the serological aspects since Paul and Bunnell discovered the presence of relatively high titers of sheep red blood cell antibodies in the serum of patients suffering from the disease[2]. In reviewing the literature with particular reference to the serological aspects, sporadic reports are found of cases with a transiently positive Wassermann. Parkes Weber[3,4] reports two cases with a positive Wassermann, a third with a negative Wassermann and positive Meinicke, and a fourth with a positive Wassermann and a positive Meinicke, and finally, one case with a positive Wassermann and a negative Meinicke. In all of these cases, the Wassermann and Meinicke became completely negative at some later date in convalescence. Tidy, in his review[1], mentions the transiently positive Wassermann as occurring occasionally, particularly, in the epidemic febrile type. In addition, Redford and Rolleston[5] report two cases with a positive Wassermann reaction in a mother and daughter, but from the clinical reports there is strong reason to suspect the actual presence of syphilis in both patients, especially since the Wassermann reaction remained positive during the entire period of observation. All of these cases were observed before the discovery of sheep cell antibodies in the serum of patients with infectious mononucleosis.

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The observations of Paul and Bunnell have been amply confirmed by subsequent workers (Rosenthal and Wenckebach[6], Davidson[7], Stuart et al.[8], Butt et al.[9], Van Ravenswaay[10]). In brief, the test consists in titrating the patient's inactivated serum against a suspension of sheep erythrocytes. Slight confusion exists in the reports because sheep cell antibodies may occur in low titer in normal human sera, and also because of differences in technic used by different investigators[8]. However, with the original technic of Paul and Bunnell, titers above 1:16 are of diagnostic significance. This is demonstrated in table 1, which summarizes two studies on large series of presumably normal individuals or patients suffering from diseases other than infectious mononucleosis.

TABLE 1

INVESTIGATOR	NUMBER OF CASES EXAMINED	PERCENTAGE OF NORMAL INDIVIDUALS WITH TITERS OF				
		Less than 1:4	1:4	1:8	1:16	13:2
Bernstein[11]	300	29	32	25	14	0
Butt and Foord[9]	412	81		19	Only 1 case	0

Davidsohn[12, 13] had previously observed the occurrence of high titers of sheep cell antibodies in patients injected with horse serum but not in cases of allergy to horse serum[10]. Absorption experiments show that these antibodies are not identical with those occurring in infectious mononucleosis. Thus Stuart et al.[14], have demonstrated that the sheep cell lysins were completely or almost completely absorbed by guinea-pig kidney in cases of horse serum sickness, whereas the sheep cell titer was not significantly reduced by absorption in infectious mononucleosis. This does not necessarily imply, however, that the antibodies in infectious mononucleosis do not belong to the Forssman group. Thus, Landsteiner and others have shown that even though this group of antigens has in common the ability to stimulate sheep

red blood cell antibody formation, they may exhibit wide variations in their antigenic specificity as revealed by absorption experiments[15].

The question now arises whether or not any connection exists between the occurrence of a positive Wassermann test and a positive heterophile sheep cell agglutination test in infectious mononucleosis. The present author has recently had the

TABLE 2

	DAY OF DISEASE					
	9th	10th	16th	21st	48th	70th
Total white cell count	15,100	11,000		9,600	5,200	5,600
Polymorphonuclears						
Neutrophilic	25%	23%		14%*	29%*	42%
Eosinophilic					3%	3%
Basophilic	1%	1%				
Lymphocytes	67%	73%		81%	65%	50%
Small	21	25		29	60	48
Large	18	22		0	5	2
Atypical	28	26		52	0	0
Monocytes	6%	3%		5%	3%	5%
Hemoglobin		98%			104%	102%
Red blood cells/cu. mm.					5,000,000	5,100,000
Paul-Bunnell		1:8	1:32	1:32	1:4	1:4
Wassermann (Modification used by N. Y. State Dep't of Health)		4 plus	4 plus	4 plus	neg.	neg.
Kline		neg.	neg.	neg.	neg.	neg.
Widal			neg.			

* (5% band.)
† (2.5% band.)

occasion to treat a patient with infectious mononucleosis from onset to convalescence, and has taken advantage of this opportunity to study the case especially from that point of view. A brief clinical history follows:

CASE REPORT

S. Z., white male, 41 years of age became acutely ill on June 3, 1936, with marked prostration, dizziness and temperature of 103 F. There were no other

subjective complaints. Examination revealed no significant physical findings except a low blood pressure of 90/70 and a relatively slow pulse rate of 80 per minute. This condition continued. A daily search for skin rash and rose spots was negative. The spleen was not palpable and the lymph nodes were not enlarged or tender. On the fifth day, the patient complained of sore throat and buzzing in his ears, the throat showed slight injection but this disappeared within two days. The fever continued, and after nine days, a blood count was taken and a picture typical of infectious mononucleosis was obtained. Blood specimens were then taken at intervals for the Wassermann, Kline, and Paul-Bunnell tests, as well as for repeated counts. The results are given in table 2. The subsequent clinical course was uneventful but characterized by a persistent weakness, with very slow recovery. The temperature fell by lysis reaching normal by the second week. After six weeks, the lymphocytes began to decrease in number and eosinophiles made their appearance. At present (April, 1937), the patient is well, although the blood count has not yet entirely returned to normal.

Clinically, it is interesting to note the relatively advanced age of the patient, and also the complete absence of palpable or tender lymph nodes or splenomegaly throughout the course of the disease. This is unusual although it has been observed before[16, 17]. It suggests the possibility that many so-called "influenza" and "grippe" cases of a lymphotropic nature, with prolonged convalescences belong to this disease and are overlooked because of failure to make proper blood studies. In these cases, moreover, where lymph node enlargement is not evident, the mononucleosis may be excessive and the fever and prostration marked, as in the case reported.

From the serological standpoint, this case offers several observations of interest. It shows once more the variation in titer of sheep cell antibodies during the course of the disease, with the peak at the 2nd and 3rd week. Whereas the absolute value of the titer at its peak was relatively low in comparison with the minimum requirement of a titer of 1:16 for a positive report, the fall in titer with convalescence leaves no doubt as to its significance. Thus the Paul-Bunnell test, like the Widal, should be repeated at intervals during the course of the disease, if it is to yield the maximum information.

The Wassermann reaction was positive at the height of the disease, but became negative as the patient recovered, showing

a parallelism in the appearance and disappearance of the Wassermann reaction and the Paul-Bunnell test. That the two antibodies are not identical, however, was shown by the fact that after the patient's serum was treated with sheep cells, the Paul-Bunnell test became negative yet the Wassermann remained positive. Especially worthy of note was the observation that the Kline test was persistently negative in the face of the positive Wassermann. In this connection, Wiener has performed parallel Wassermann and Kline tests on a series of more than 2000 individuals amongst patients with and without syphilis[18] and not a single instance was encountered among the syphilitics in which a negative Kline was accompanied by a positive Wassermann, although it was not unusual to find the Kline test positive and the Wassermann negative. In the rare instances in which a negative Kline was obtained in the face of a positive Wassermann, the patient did not have syphilis. Hence, the antibodies in infectious mononucleosis are not identical with those causing positive Wassermann reactions in syphilis, or else the Kline test should also have been positive. The positive Wassermann reaction must be attributed to the presence in the antigen used for that test, of some specific binding group not present in the Kline antigen.

Referring to the heterophile nature of the antibodies in infectious mononucleosis and their similarity to the Forssman antibodies, it is interesting to note that there have been reports which show that Forssman antibodies could be a cause of positive Wassermann reactions provided that the antigen used was prepared from the tissues of an animal in the Forssman group such as guinea-pig and horse[19,20]. This may account for the positive Meinicke reaction obtained by Parkes Weber in infectious mononucleosis, since horse heart is used in preparing the antigen used for that test. Moreover, since his Wassermann tests were probably performed in the same laboratory, it is possible that the horse heart antigen was also used for the Wassermann test. In the present study, the antigen used in the Kline test was prepared from dried beef heart (Difco) in accordance with Kline's direction. Since the ox belongs to the

group of Forssman negative animals, on the above assumption, a negative Kline reaction was to be expected. The antigen for the Wassermann test was obtained from the New York City Department of Health. Since this antigen is also supposed to be prepared from ox heart, the positive Wassermann reaction was somewhat unexpected. At the present time, we cannot account for this discrepancy between the two tests except by assuming either (1) that the beef heart used was adulterated with tissues of another animal or (2) that it was due to some difference in the preparation of the two antigens.

SUMMARY AND CONCLUSIONS

A case of infectious mononucleosis of the febrile type is described, including the pertinent hematological and serological findings. Clinically the case was of interest because the complete absence of enlargement of lymph nodes or spleen, coupled with the age of the patient, could readily have led to a diagnosis of "influenza" or "grippe" if proper blood studies had not been made. Serologically, a positive Paul-Bunnell test, a positive Wassermann (New York State Department of Health modification) associated with a negative Kline test, was obtained. As the patient recovered, both the Paul-Bunnell and Wassermann tests became negative. This seems to point to a similarity in the mode of formation of the antibodies concerned.

The author is indebted to Dr. A. S. Wiener for the performance of all the laboratory studies reported here and for his valuable criticisms and suggestions.

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VENOUS AND PERIPHERAL RED BLOOD CELL VALUES*

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Several investigations of the quantity of hemoglobin, of the number of red blood cells, and of the volume of packed cells in venous blood have been reported in recent years. The results of these determinations have been proposed as standards with the idea that values for venous and peripheral blood are practically identical. Some evidence for this belief was presented by Wintrobe[7] in 1930 who concluded from a review of the literature and from his own observations that red blood cell counts obtained from the finger tip were not appreciably different from those made on venous blood when care was taken to secure a free flow of blood from the finger prick. Price-Jones, Vaughan and Goddard[5] also reported in 1935 that there was no significant difference in the averages of hemoglobin and the number of red blood cells in samples of venous and peripheral blood from 100 male adults.

Similar investigations on young infants have not been so conclusive. Lucas and co-workers[3] in 1921 compared a series of blood samples taken from the longitudinal sinus of infants varying in age from one to 12 days with another series taken by puncture from the heel. They found that after the first day of life both the amount of hemoglobin and the number of red cells were slightly higher in the blood from the sinus than in the peripheral sample. Haden and Neff[2] in 1924 found, however, in 6 infants

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This investigation was undertaken at the suggestion of Dr. A. H. Washburn and carried out with the help of Dr. John A. Anderson and Dr. M. Elizabeth Downing who secured many of the venous blood samples.

ranging in age from 5 to 24 days that the red blood cell counts were uniformly higher in the peripheral blood than in the blood from the longitudinal sinus. They believed this difference was probably related in some way to the increased volume of the individual cells in the blood of infants of this age. A search of the literature has not revealed any adequate data for older infants and children. We have recently reported a number of analyses of venous blood of infants, children and adults. In that paper[4] it was suggested that valuable information could be obtained by following the blood picture of individual children through the years of their development. At the present time we are conducting such a study on peripheral blood samples. Comparison of the values for this group with those which we have obtained on venous blood can be made only if peripheral and venous blood samples of infants and children are shown to be in close agreement. Our investigation of this problem is therefore presented.

SUBJECTS AND METHODS

The subjects were obtained from the same sources which were described in the two previous papers.[1,4] They include 30 adults of both sexes; 30 boys and girls between the ages of 2 and 14 years; 30 young children of both sexes from one month to 19 months of age and 30 infants ranging in age from 30 minutes after birth to 19 days.

Venous blood samples were secured from an external jugular or cubital vein of the young infants, and from a cubital vein of the older children and adults. The application of a tourniquet was frequently necessary in order to secure the samples from the arm vein. However the duration of application of the tourniquet was kept at a minimum. Heparin was used as the anticoagulant for both venous and peripheral blood samples. Immediately after the venous sample was secured, blood was collected from the heel of the infants and from the finger tip of the older children and adults. A puncture wound deep enough to give a free flow of blood was made with a spring lancet and approximately 0.5 cc. of blood was collected in a small vial.*

Duplicate determinations of hemoglobin were made on each sample with a

* Three drops of a 1 per cent solution of heparin were run into flat bottomed, wide mouthed vials with a capacity of about 1 cc. By evaporation in an oven at 100°C. a thin film of anticoagulant was deposited on the sides and bottom of the vials.

Hellige hemoglobinometer which we had calibrated by the Van Slyke-Neill oxygen capacity method. Red blood cell counts were made on at least two dilutions from each sample and the average of two counts differing by less than 200,000 cells was taken as the value for the sample. Most of the determinations of the volume of packed cells were run in hematocrit tubes which were constructed from sections of 0.2 cc. serological pipettes 10 cm. in length. After the tube was filled with blood, one end was sealed by forcing it into a hole which was bored halfway through a rubber stopper. Duplicate determinations were run on each sample. In the latter part of the study hematocrit tubes as described by Smith[6] were substituted. These possessed the same degree of accuracy as those previously used, but were much easier to manipulate.

A series of 10 consecutive determinations on the same blood sample showed that the estimation of packed cell volume was the most accurate of the three procedures. The coefficient of variation for the hematocrit readings was 0.48 per cent; for the red blood cell counts, 1.3 per cent; and for the determinations of hemoglobin it was 2.2 per cent.

RESULTS

The averages for the quantity of hemoglobin, the number of red blood cells and the volume of packed cells in venous and peripheral blood are given in table 1. Since the determination of the volume of packed cells is the most accurate of the three procedures, the hematocrit values should be given the greatest importance in comparisons of the results. In infants under 3 weeks of age the average volume of packed cells is 3.36 cc. higher on blood from the heel than on that from the vein. In 12 cases of 30 the individual difference exceeds the average; the greatest difference of 7.8 cc. is found in a baby 40 minutes after birth. In only two members of this group is the volume of packed cells in the venous blood slightly higher than in the peripheral blood. Statistical analysis by the standard error of the difference of the means reveals that this difference is great enough to be considered as possibly significant only in young infants. In children between the ages of one and 19 months the volume of packed cells in peripheral blood is slightly but not significantly higher than in venous blood. Averages of the samples from the two sources in older children and adults are almost identical.

The difference between venous and peripheral blood samples in the first three weeks of life is probably attributable to several

TABLE 1

	MEAN	STANDARD DEVIATION	STANDARD ERROR	COEFFICIENT OF VARIATION	DIFFERENCE OF MEANS	STANDARD ERROR OF DIFFERENCE
Hemoglobin						
30 adults						
Venous	15.04	0.98	0.18	6.5	0.06	0.27
Peripheral	15.10	1.08	0.20	7.1		
30 children, 2–14 years of age						
Venous	13.68	0.79	0.14	5.7	0.03	0.21
Peripheral	13.65	0.83	0.15	6.1		
30 infants, 1–19 months of age						
Venous	12.20	0.96	0.17	7.8	0.55	0.25
Peripheral	12.75	0.94	0.17	7.3		
30 infants, 30 minutes to 19 days of age						
Venous	18.18	2.22	0.40	12.1	0.70	0.57
Peripheral	18.88	2.22	0.41	11.2		
Red blood cells						
30 adults						
Venous	4.880	0.382	0.070	7.8	0.037	0.107
Peripheral	4.917	0.444	0.081	9.0		
30 children, 2–14 years of age						
Venous	4.586	0.273	0.050	5.9	0.012	0.068
Peripheral	4.598	0.257	0.047	5.5		
30 infants, 1–19 months of age						
Venous	4.096	0.337	0.062	8.2	0.051	0.085
Peripheral	4.147	0.317	0.058	7.6		
30 infants, 30 minutes to 19 days						
Venous	4.920	0.498	0.091	10.1	0.264	0.131
Peripheral	5.184	0.519	0.095	10.0		
Volume of packed cells						
30 adults						
Venous	44.60	3.27	0.60	7.3		
Peripheral	44.60	3.26	0.60	7.3		
30 children, 2–12 years of age						
Venous	39.59	2.56	0.47	6.4	0.25	0.68
Peripheral	39.34	2.68	0.49	6.8		
30 infants, 1–19 months of age						
Venous	36.21	2.84	0.52	7.8	0.62	0.74
Peripheral	36.83	2.88	0.53	7.8		
30 infants, 30 minutes to 19 days of age						
Venous	54.92	6.19	1.13	11.0	3.36	1.61
Peripheral	58.28	6.30	1.15	10.8		

factors. As suggested by Haden and Neff[2] one of these may be the large size of the blood corpuscles. This fact is shown in table 2; the average volume of the red blood cells of the infants under 19 days of age is much larger than that of any of the other groups. However in this group as well as in all the others there is no appreciable difference in size between the cells of venous and of peripheral blood. The lability of the vasomotor control in young infants may also be concerned in producing capillary stasis with some packing of the large red cells in the peripheral vessels.

TABLE 2

MEAN CORPUSCULAR VOLUME (CUBIC MICRONS)*

AGE	VENOUS	PERIPHERAL
30 infants, 30 minutes to 19 days	111.6	110.4
30 infants, 1 to 19 months	88.4	88.8
30 children, 2 to 14 years	86.3	85.5
30 adults	91.3	90.7

* The mean corpuscular volume in cubic microns or cubic centimeters $\times 10^{-12}$ is a term proposed by Wintrobe[3] to represent the volume of the individual cell. It is obtained by dividing the volume of packed cells in cubic centimeters per thousand cubic centimeters of blood by the red blood cell count in millions per cubic millimeter.

SUMMARY

These averages from blood samples of 120 subjects at various ages seem to indicate that after the first three weeks of life values for the quantity of hemoglobin, the number of red blood cells and the volume of packed cells in venous and peripheral blood are in close agreement and can be used interchangeably.

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SPONTANEOUS RUPTURE OF THE MYOCARDIUM*

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This type of pathological lesion has been described by Krumbhaar and Crowell[1] as "the event of a lifetime" in the experience of those persons engaged in the study of morbid anatomy. Their presentation of the subject will remain a classic for many years to all who become interested in the problem, and we have nothing to add therefore to the pathological concepts which they have so well interpreted.

Stewart[2] presented an instance of this lesion in 1932 and added electrocardiographic tracings to the accumulated data concerning its prodromal manifestations. It is our impression that in the present age of "coronary medicine" the possibility of spontaneous rupture of the myocardium has not been emphasized sufficiently in view of the absolute fatality which the spontaneous lesion entails. For this reason, and because we have evidence which will aid the clinician in an appreciation of the prodromal phenomena of this condition, this presentation is considered timely.

CLINICAL AND PATHOLOGICAL NOTES

Patient, C. F., a white female, age 79 was admitted in 1929 for the second time to the Norristown State Hospital. Relevant facts of her history caused a diagnosis of psychosis with mental deficiency to be made and it was also noted that she had an old left hemiplegia and arteriosclerosis.

The Kahn test and Kolmer's modification of the Wassermann reaction were both negative on her blood. When admitted, urine examination showed a faint trace of albumin with a sp. gr. of 1.021. The admitting physician noted the following on cardiac examination,—"Apical impulse palpable in the 5th interspace just external to the mid-clavicular line. Area of cardiac dulness is normal. Sounds of fair muscular quality. No murmurs."

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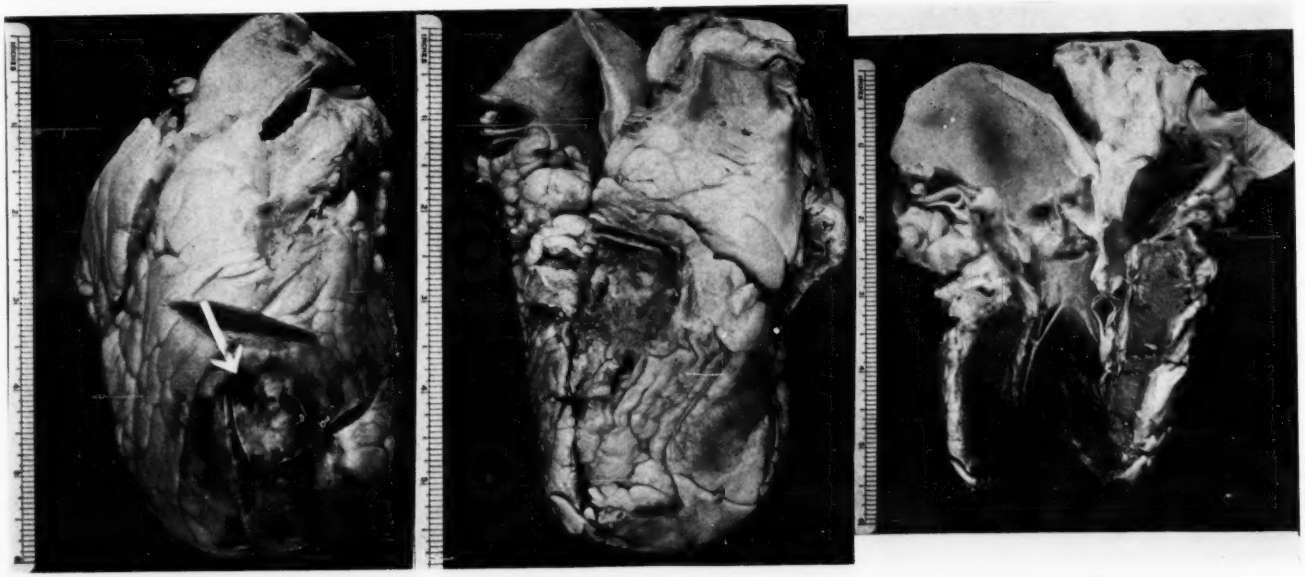

A B C

FIG. 1. A. Specimen showing the marked increase in sub-epicardial fat, point of ventricular rupture marked by arrow and the area of infarction which measured 3 x 7.5 cms.

B. Portion of the right coronary opened to show the pipe stem sclerotic character of the vessels.

C. Left ventricle opened showing the large gray-red ante mortem thrombus covering the point of rupture on the internal aspect of the ventricle.

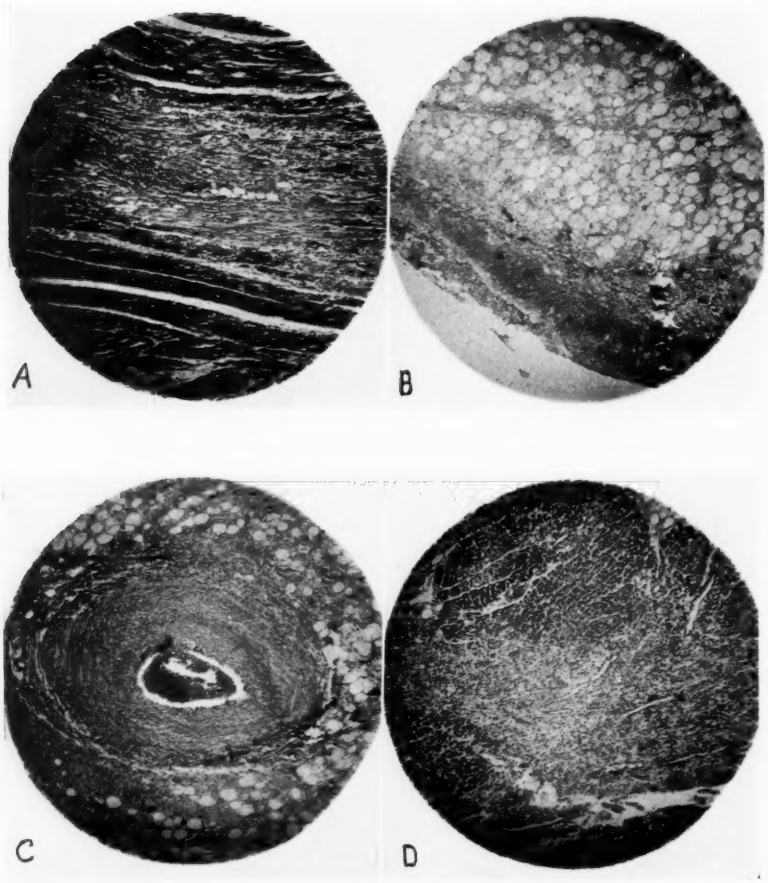

FIG. 2. A. Portion of the myocardium showing a small area of fatty degeneration and fibrosis. Apparently occlusion of the small arterioles preceded the massive terminal occlusion.

B. Marked increase in the sub-epicardial fat with pericardial collection of fibrin and red cells shown in the lower portion, incident to pericardial clot formation.

C. Portion of the anterior descending branch of the left coronary showing the atherosclerotic narrowing of the lumen and terminal occlusion by thrombus.

D. Section of myocardium at point of terminal infraction, showing necrosis of the muscle and portion of the actual tear at point of rupture.

Cardiac examination in 1934 showed her apex beat was in the 5th interspace at the mid-clavicular line. Sounds were of poor quality, rhythm was regular. There was an apical systolic murmur and the aortic 2nd sound was greater than the pulmonic 2nd sound. Pulse was 88 per minute.

In early Spring of 1936 she was found to have a fracture of the anatomical neck of the left humerus and apparently led a guarded institutional life until her last illness.

At this time notes by the attending physician were as follows,—"June 7, 1936. Patient indicated she had abdominal pain. She was unable to articulate clearly and when asked where she felt sick, pointed to her abdomen. The abdomen was scaphoid and wasted but there was muscular tension and evidence that the patient was constipated. No localized tenderness was elicited on palpation. Lungs were hyper-resonant without râles. Mucus membranes were anemic. Heart borders were within normal limits, sounds were of poor quality without noticeable murmurs. Temperature was 98.4, pulse 96, respirations 26. She appeared very weak.

"The patient was put to bed, given extra nourishment and seemed to improve. On the 15th she indicated that she would like to sit in a chair which was permitted for ½ hour. After supper she became active, sat up in bed and tried to climb out and suddenly fell back on her pillow and expired."

Post-mortem examination of the body was made 15 hours after death. On removing the chest plate the pericardium was noted to be bluish gray in color. When the pericardial sac was opened the heart was found to be completely surrounded by a firm, jelly like, dark red blood clot which formed a cast of the cardiac surface and pericardial sac although it was not adherent. The clot was deficient only in the apical area, suggesting that the heart beat for a short interval before compression or "tamponade" restricted diastole and contributed to a cessation of its action.

The heart weighed 360 gms. There was a marked increase in the sub-epicardial fat. The apex and anterior surface of the left ventricle presented an irregular area of infarction which measured 3 × 7.5 cms. (Fig. 1, A). On the pericardial surface of this area the point of rupture of the myocardium is shown. Coronary vessels were tortuous and palpably sclerotic (Fig. 1, B). Their lumens were open on section but the anterior descending branch could not be completely probed (Fig. 2, C). The myocardium in general showed great degeneration (Fig. 2). The site of rupture on the endocardial surface of the left ventricle was covered by a grey red ante-mortem thrombus which was firmly adherent (Fig. 1, C).

Other anatomical findings were mild congestion of the lung bases, moderate congestion of the liver and kidneys with chronic nephritis, atherosclerosis of the aorta with calcific plaques in the aortic arch and muscular atrophy associated with an old left hemiplegia. Death resulted from the spontaneous rupture of the left ventricle with hemorrhage into the pericardial sac subsequent to the attempt at repair of an infarction of the anterior surface of the left ventricle.

COMMENT

Because the clinician had no suspicion of the true picture in this case we were curious as to whether any studies had been made which might be helpful in directing attention to the underlying pathological process. Tabulation of the blood pressure

TABLE 1

BLOOD PRESSURE RECORD

	1929	1934	1934	Infarct	1936 (JUNE 7)
Systolic	148	188	184		120
Diastolic	70	98	104		70

The abrupt fall in level of the blood pressure coincides with clinical symptomatology which autopsy proved to be due to infarction of the myocardium.

HEMOGRAMS OF INFARCTION WITH SUBSEQUENT RUPTURE

	GRAMS HEMOGLOBIN	MILLIONS R. B. C.	THOUSANDS W. B. C.	MULTIPLE INDEX	SCHILLING INDEX	NEUTROPHILS							LYMPHOCYTES					MONOCYTES	EOSINOPHILS	BASOPHILS	MATURE NEUTROPHILS PER CU. MM.
						Myeloblast	Prom'locyte	Myelocyte	Metamyelocyte: Juvenile	Metamyelocyte: Stab	Segmented	Total	Lymphoblast	Large	Medium	Small	Total				
Normal	15.4	5.0	7.5	1	1–16	0	0	0	0	4–16	64	68	0	5	1	20	26	4	1	1	4800
9– 6–29	10.1	4.1	5.0	?	?	0	0	0	0	?	?	55	0	0	0	0	41	3	1	0	?
6– 7–36	....	....	....	....	....	....	....	....	....	*Infarction*	....	....	....	....	....	....	....	....	....	....	....
6– 8–36	13.5	4.9	26.9	24	1.5	0	0	0	0	52	34	86	0	3	2	0	5	9	0	0	9146
6– 9–36			16.6	27	1.7	0	0	0	0	51	29	80	0	2	2	4	8	12	0	0	4814
6–15–36	....	....	....	....	....	....	....	....	....	*Myocardial rupture*	....	....	....	....	....	....	....	....	....	....	....

Hemographically there is a neutrophilic leukocytosis and degenerative left shift of 24 and 27 multiples. The neutrophilic leukocytosis is maintained, but the actual number of segmented neutrophils per cu.mm. is cut to normal and the total white cell count is lowered yet the multiple index (toxic index) is not reduced. This conforms to the Type I hemogram of Crocker[3]. Findings of this type in the blood picture point to physical illness and should cause one to question the report of a normal temperature.

levels over the period of hospitalization and of the blood counts, particularly those analyzed by hemographic methods (Table 1), reveals that the fall in blood pressure, leukocytosis, degenerative left shift of the neutrophils and increase in the multiple index to

24, all coincide with the date of clinical symptoms which were proven to be due to infarction of the myocardium.

This case occurred in a psychotic patient. Krumbhaar has noted the high incidence of this entity among the mentally ill. Including this case the records of the Norristown State Hospital show that spontaneous rupture of the heart has been noted 4 times in 2096 autopsies.

This lesion is an accident which while rare is seen in the left ventricle of the aged, arteriosclerotic individual. While the subepicardial fat is increased the rupture is usually due to coronary artery disease either from gradual fibrotic occlusion or sudden thrombosis of a main or lesser branch.

In Stewart's case an argument with another patient preceded the rupture while in this instance the patient tried to get out of bed. Thus anger and physical effort both predispose to rupture of the damaged muscle. In our opinion, the resultant pericardial hemorrhage increases in size and restricts the heart's ability to fill during diastole. This "tamponade" so limits the cardiac output that the vital cerebral centers which have been functioning at their minimal oxygen requirement are quickly rendered anoxemic and death follows.

The possibility of spontaneous rupture of the myocardium should always be visualized when meeting instances of cardiac insufficiency in the aged. The electrocardiogram is a valuable diagnostic agent at Stewart has shown. Our findings suggest that hemographic analysis of the blood picture when associated with a fall in blood pressure may serve also to direct the clinician's attention to a toxic focus in the myocardium; always with the provision that such other conditions as ectopic pregnancy, ruptured kidney, incarcerated hernia and dissecting aneurysm of the aorta may be eliminated clinically.

SUMMARY

1. A case of spontaneous rupture of the heart is reported with hemographic analysis of the blood picture.

2. Hemography may be of aid to the clinician in the identification of the prodromal manifestations of spontaneous rupture.

3. It is probable that in this case multiple small infarctions of the musculature preceded the final infarction and subsequent rupture.

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THE HISTOGENESIS, CLASSIFICATION AND IDENTIFICATION OF THE CELLS OF THE BLOOD AND MARROW BASED ON CULTURES AND HEMATOLOGIC STUDIES OF HUMAN MARROW AND BLOOD*

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In the course of research on the diagnostic value of marrow obtained by sternal puncture[1], on the culture of sternal marrow[2], and in the preparation of an Atlas of Hematology[3], it was necessary to decide on a uniform nomenclature for the cells of the blood and marrow and to devise a series of definitions and a system of cell identification which were sufficiently clear so that different technicians counting the same preparations could duplicate each others' results with reasonable accuracy.

NOMENCLATURE

The need for a standardized nomenclature is illustrated by table 1 which gives the terminology chosen and the other names which have been applied to the same cells. The definitions of the recommended terms are indicated in the tables of cell identification. It is evident from this table that much of the confusion in hematologic literature is due to the terminology. Authors have used terms without defining them clearly so that others can be certain to use them in the same way, and many terms have been coined to describe the same cell type. Actually, cells undergo continuous changes in the process of maturation and it is possible to divide them into a few stages, just as people may be divided into children or adults, or into a great many stages (newborn infants, preschool or school age, adolescents, etc.). Any classification is arbitrary but some subdivision is necessary.

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TABLE 1

NOMENCLATURE

NAME OF SERIES	RECOMMENDED NAME	NAMES WHICH HAVE BEEN APPLIED TO THE SAME CELL
Lymphocyte	Lymphoblast	Myeloblast, hemocytoblast, lymphoidocyte, stem cell, lymphocyte
	Prolymphocyte	Large lymphocyte, pathologic large lymphocyte, atypical leukocytoid lymphocyte, monocyte
	Lymphocyte	Small, medium, or large lymphocyte, normal lymphocyte, small, medium or large mononuclear
Monocyte	Monoblast	Myeloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell, immature monocyte
	Promonocyte	Premonocyte, hemohistioblast, immature monocyte
	Monocyte	Large mononuclear, transitional, clasmatocyte, endothelial leukocyte, histiocyte, resting wandering cell
Granulocyte (Myeloid)	Granuloblast	Myeloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell
	Progranulocyte S*	Promyelocyte I, myelocyte A, myelocyte, non-filament, class I
	Progranulocyte A	Promyelocyte II, leukoblast, basophil myelocyte, myeloblast, premyelocyte
	Granulocyte	Myelocyte, myelocyte B, non-filament, class I
	Metagranulocyte	Metamyelocyte, juvenile, myelocyte C, non-filament, class I
	Rhabdocyte	Staff cell, stab cell, band cell, non-filament, class I, rod nuclear, polymorphonuclear
	Lobocyte	Segmented neutrophil, polymorphonuclear, filamented, class II, III, IV or V
Plasmacyte	Plasmablast	Myeloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell, lymphoblastic plasma cell
	Proplasmacyte	Türk cell, Türk irritation form, lymphoblastic or myeloblastic plasma cell
	Plasmacyte	Plasma cell, Unna's plasma cell, Marschalko plasma cell, plasmacytoid lymphocyte

* Any basophil from the progranulocyte to the lobocyte is sometimes referre to as a *mast cell.*

TABLE 1—*Concluded*

NAME OF SERIES	RECOMMENDED NAME	NAMES WHICH HAVE BEEN APPLIED TO THE SAME CELL
Erythrocyte	Karyoblast	Megaloblast, myeloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell promegaloblast, basophilic normoblast, primitive erythroblast
	Prokaryocyte	Erythroblast, megaloblast, orthochromatic normoblast, basophilic normoblast, polychromatophilic normoblast, macronormoblast, macroblast
	Karyocyte	Normoblast, pronormoblast, macronormoblast, erythroblast, polychromatophilic normoblast
	Metakaryocyte	Normoblast
	Reticulocyte	
	Akaryocyte	Erythrocyte, red blood cell, erythroplastid, normocyte
Thrombocyte	Megalokaryoblast	Megakaryoblast
	Promegalokaryocyte	Promegakaryocyte
	Megalokaryocyte	Megakaryocyte
	Platelet	Thrombocyte, thromboplastid
	Disintegrated cell	Senile cells, smudge, basket cell, smear cell, degenerated cell

The most practical classification should have the fewest subdivisions compatible with diagnostic and descriptive accuracy. The terminology should be descriptive and clearly defined. These ideals have been kept in mind in choosing this nomenclature.

The basis for classification and nomenclature of the cells of the blood and marrow should be the histogenesis of the cells. Most hematologists will agree with the major lines of cell development as depicted in figure 1 with certain reservations which will be discussed a little later. Rather than coin an entirely new nomenclature, the terms in current use which seemed most accurately descriptive have been selected as the preferred terms and have been redefined in the Atlas[3] with such definite criteria that it is hoped that anyone using these criteria will classify the same cell in the same way.

In the case of the erythrocyte and granulocyte series, however, a new nomenclature has been introduced but in this paper the old

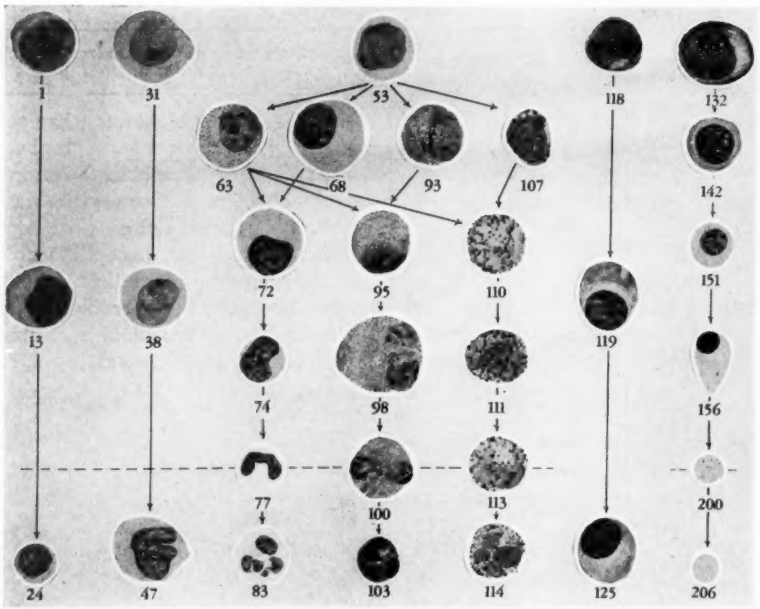

FIG. 1. In this figure, a typical example of each stage in the maturation of each series of cells is pictured. The number under the cell refers to the number of the same cell in the Atlas where it will be found pictured on a larger scale, showing the finer detail of its structure. There will also be found a detailed description of the characteristic morphology and illustrations depicting most of the variations in the appearance of this cell type. A dotted line separates the cells normally found in the blood from those which are found normally only in the marrow or lymph nodes after fetal life. The arrows indicate the lines of maturation. The least mature cell is given at the top and the most mature cell at the bottom of the plate. In the lymphocyte series, the lymphoblast (1) and prolymphocyte (13) are capable of mitotic division and the lymphocyte (24) of amitotic division. In the monocyte series, the monoblast (31) and promonocyte (38) are capable of mitotic division, while the monocyte (47) probably does not divide. In the granulocyte series, the granuloblast (myeloblast) (53), the neutrophil, eosinophil and basophil progranulocytes S (promyelocytes I) (68, 93, 107) and the progranulocytes A (promyelocytes II) (63) are capable of mitotic division but most divisions occur normally in the progranulocyte A. Amitotic division has not been observed in any cell of this series nor have the granulocytes (myelocytes) (72), metagranulocytes (metamyelocytes) (74), rhabdocytes (staff cells) (77) or lobocytes (polymorphonuclears) (83) been observed to divide at all. In the plasmacyte series, the plasmablast (118) and proplasmacyte (122) may undergo either mitotic or amitotic division but the

term most nearly equivalent to the new term is given in parenthesis. It seemed necessary to introduce a new nomenclature for the erythrocyte and granulocyte series because of the disagreement in the definitions and the inappropriateness of the terms in current use. To make certain that they were understood as intended, anyone using the old terms would have to define them each time they were used. The term *normoblast* exemplifies the inappropriateness of the old terms. It is a combination of a Latin and Greek root which should mean a normal stem cell since the termination-*blast* is ordinarily employed only for the most immature cell of a series. The cell, however, is neither a stem cell nor a normal cell of the blood.

The derivation of the new terms for the erythrocyte series is from the Greek word meaning *nucleated.* It would have been a little more logical to include the syllables *erythro* between the karyo- and the final syllable, thus making *karyoerythroblast, prokaryoerythrocyte,* etc., but this makes the name unduly long and it seemed better to omit these syllables and have it understood that these are cells of the erythrocyte series.

The old term *myeloid series* means marrow-like cells. This is a misnomer because they are cells forming an integral part of the marrow. Furthermore, this does not differentiate them from cells of the monocyte, plasmacyte or erythrocyte series which are also found in normal marrow. On the other hand, the term *granulocyte series* has come into current use for all cells of this group and seems much more logical. The letters

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plasmacyte (125) only amitotic division. In the erythrocyte series, the karyoblast (megaloblast) (132) alone undergoes mitotic division, the prokaryocyte (erythroblast) (142) and karyocyte (pronormoblast) (151) undergo amitotic division, while the metakaryocyte (normoblast) (156), reticulocyte (191) and akaryocyte (non-nucleated erythrocyte) (206) do not divide. The histogenesis of the thrombocyte series is not illustrated because the cells are too large to go on the page. The earliest cell is the megalokaryoblast, the next stage is the promegalokaryocyte, and the third stage is the megalokaryocyte which forms the platelets by splitting off of fragments of the cytoplasm. These cells are described on page 104 and the platelets (308–309) and megalokaryocytes (310–311) are illustrated. The first two stages are capable of mitotic division. Only the platelets are found in the blood stream.

S and A after the progranulocytes (promyelocytes) have been substituted for the I and II to avoid a suggestion of sequence. S applies to cells which have *s*pecific granulation, i.e., neutrophil, eosinophil or basophil, and A applies to cells which have *a*zurophil granulation or are *a*granular. The terms *rhabdocyte* and *lobocyte* should really be *rhabdogranulocyte* and *lobogranulocyte* but these seemed unduly cumbersome. The derivation of the term *rhabdocyte* is from the Greek word meaning "curved rod, stick or wand" which well describes the shape of the nuclei of these cells. The term *lobocyte* is from the Greek word meaning "lobed" and should also suggest the typical lobed or segmented character of the nuclei of these cells much better than the cumbersome old term *polymorphonuclear*. The use of these terms avoids the confusion arising from the use by some authors of the term *polymorphonuclear* to include the rhabdocytes (staff cells).

HISTOGENESIS

Figure 1 illustrates the author's conception of the histogenesis of the cells of the blood and marrow. It is based on a study of the literature evaluated in the light of a study of many thousand blood smears, over four hundred sternal marrow smears, and over four hundred separate experiments on the culture of human marrow. The evidence accumulated is too extensive to present here in detail but much of it will be found in the atlas of hematology.[3] These studies lead to a classification agreeing with that of all major students of the subject in all essential details except those discussed below.

Note in figure 1 that the first cells of each series are almost identical in appearance. The monophyletists or unitarians, including Downey[4], Kato[5], Ferrata[6], and Pappenheim[7], believe that these cells are actually identical and use the terms *myeloblast* (Downey), hemacytoblast (Ferrata), or lymphoidocyte (Pappenheim) for this cell.

The dualists believe that the granuloblast (myeloblast), monoblast, lymphoblast, and plasmablast, as pictured, are actually one and the same cell which is capable of giving rise to any of the leukocytes but that the karyoblast (megaloblast) is a distinct cell type, capable of giving rise to cells of the erythrocyte series only. The leading exponents of this school are Ehrlich[8], Naegeli[9], Schridde[10], Piney[11], and Helly[12].

The trialists, among whom are Schilling[13] and Rosenthal[14], differ from the dualists only in believing that the monocyte series is derived from a reticulo endothelial cell of the spleen (not the monoblast here depicted). They agree with the dualists that the other leukocytes are derived from a single stem cell,

usually called a myeloblast, and the erythrocytes from a third stem cell, usually called a megaloblast.

The extreme polyphyletists, represented by Sabin[15] and her students, believe each of the series is derived from a separate stem cell somewhat as here pictured.

The author believes that both the monophyletists and polyphyletists are right. It is absolutely certain that all of the cells are originally derived from a fertilized ovum and probably much later in embryonic life there is a single cell capable of giving rise to either a granuloblast (myeloblast), monoblast, lymphoblast, plasmablast, or karyoblast (megaloblast). This single cell probably is either an embryonic reticulum cell or an embryonic endothelial cell. It is the belief of the author that once the stem cells have been formed, they lose the ability to differentiate into any other cell than those in the line of development shown in figure 1.

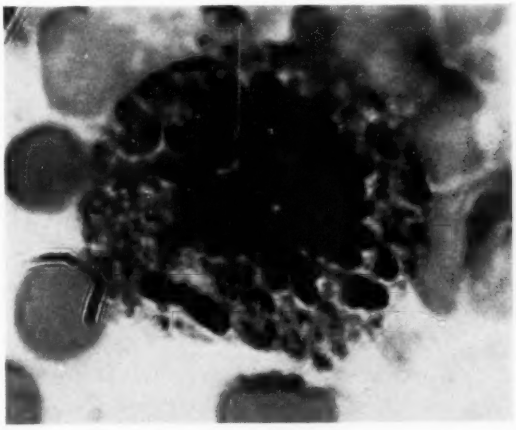

FIG. 2

The chief evidence against the monophyletic view is that no leukemias have been reported in which prolymphocytes, progranulocytes (promyelocytes), promonocytes, proplasmacytes, and nucleated red cells were all present together. Similar evidence makes the dualistic and trialistic theories difficult to accept. Furthermore, the evidence from marrow studies in leukemias and marrow cultures from normal sternal marrow is that mitotic division after fetal life occurs not in some fixed tissue cells such as a reticulum cell or endothelial cell but chiefly in the lymphoblast and prolymphocyte stage, in the granuloblast (myoblast) and progranulocyte (figure 2) stage, in the monoblast and promonocyte stage, in the plasmablast and proplasmacyte stage, and in the karyoblast (megaloblast) stage (figure 3). This makes unnecessary the assumption that the stem cells are being continuously formed from fixed tissues cells after fetal life.

Wiseman[16] would disagree with the lymphocyte series as here depicted since he feels that the criteria of immaturity in this series should be the degree of basophilia in the cytoplasm, but the evidence from studies of the blood and marrow of acute leukemias, infectious mononucleosis, and cultures of the blood and marrow of these diseases leads the author to agree with most other hematologists in the classification here depicted.

Most hematologists would disagree with the author in listing a separate plasmacyte series as they believe that plasmacytes are altered lymphocytes. The evidence for a separate plasmacyte series has been, in part, presented in a paper on plasmacytic leukemia[12]. Since this paper was written, cultures of blood and marrow have shown that normal blood and marrow contain cells similar to 118 and 119 which are capable of both mitotic and amitotic division and which mature into typical plasmacytes, similar to 125. No transitions

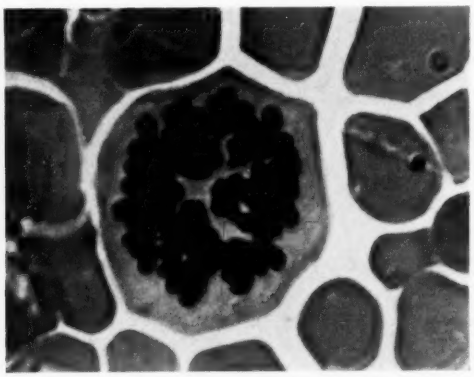

FIG. 3

have been seen in this material between a lymphocyte and any cell of the plasmacyte series.

Some hematologists, notably Downey[4], Piney[11], and Kato[5], would disagree with the erythrocyte series as shown here. These investigators believe that there are two separate series of red cell formation. One they call the megaloblast series in which they would name the cell numbered 134 in figure 1 a *promegaloblast* and consider that it develops through a megaloblast stage to a large non-nucleated cell called a *megalocyte*. The other they call the normoblast series and consider that it begins with a cell similar to the one pictured as a prokaryocyte, which they would call a *pronormoblast* or a *normoblast*, and develops through the stage of a normoblast into the erythrocyte of normal blood in post-fetal life. They believe that the megaloblast series is a method of red cell formation developing early in fetal life and not appearing as a method

of red cell formation after birth in any condition except pernicious anemia, but that in pernicious anemia, the formation of red cells reverts to that early fetal form. The chief evidence in favor of this view is the fact that the marrow in pernicious anemia and in early fetal life does contain large numbers of cells similar to 134 and that such cells are very scarce in the marrow of healthy persons or in diseases other than pernicious anemia. These authors believe that they do not occur at all in healthy marrows. Since, in the process of maturation, there is a tendency for basophilia of the cytoplasm to decrease and hemoglobin formation to increase, they reason that the cells found in pernicious anemia with a very immature nuclear structure which have already developed much hemoglobin in the cytoplasm must be more mature than cells with a nucleus similar to that shown in the cell here called a prokaryocyte which contains little hemoglobin in the cytoplasm.

However, it seems to the author unjustifiable to use the amount of hemoglobin in the cytoplasm as the criterion of the age of the individual cell since many polychromatophilic akaryocytes (non-nucleated red cells) are seen which contain practically no hemoglobin and these must certainly be more mature than nucleated red cells which contain much hemoglobin. If one uses the nucleus alone, however, as the criterion of the maturity of the cell, one can arrange a continuous series, each one differing from the neighboring cell by an almost imperceptible degree, from the most immature karyoblast (megaloblast) to the most mature metakaryocyte (normoblast) which is just losing its nucleus. Furthermore, the author has been able to find in normal marrow and in the marrow of many diseases other than pernicious anemia karyoblasts identical in all respects to those seen in the marrow in pernicious anemia although admittedly they are much less numerous and not likely to be encountered unless one looks at thousands of cells. An additional point in favor of the author's view is that administration of antipernicious anemia principle to patients with pernicious anemia results in a gradual transformation of the numerous immature karyoblasts (megaloblasts), or promegaloblasts of Downey, into typical metakaryocytes (normoblasts). This can be followed from day to day by sternal puncture in the patient or from hour to hour in marrow cultures of pernicious anemia marrow. It does not seem likely that a deficiency occurring in mature life would produce a reversion to an embryonic type of blood formation and that the administration of the deficient substance would produce a return to an adult type of erythrocyte formation. The author, therefore, agrees with Isaacs[18] and Sabin[15] in believing that the karyoblast (megaloblast) is the precursor of the metakaryocyte (normoblast) and that in the illustrations in Kato's article[5] the actual progression would be down the megaloblast series and then through the normoblast series, rather than having these two series parallel each other.

It will be noted that in each series the first step in maturation is an increase in coarseness of the chromatin structure of the nucleus. Usually this is followed by loss of nucleoli and changes in nuclear shape. In the author's opinion,

the changes in cytoplasm, such as the development of granules or of hemoglobin, decrease in basophilia, etc., are much less important criteria of the stage of development than the nuclear characteristics.

This view of the histogenesis of cells of the blood and marrow, while it seems to have the preponderance of evidence in its favor, can not be regarded as finally established until a large series of cultures have been made, beginning with a single cell, the identity of which has been conclusively established. Such cultures have not yet been made but it seems possible that a modification of the technic now used for marrow culture may permit this.

TABLE 2

NEUTROPHIL GRANULES*

NUCLEOLI	NUCLEUS	NAME OF CELL
Present	Round or oval	Neutrophil progranulocyte S (Neutrophil promyelocyte I)
Absent	Round or oval	Neutrophil granulocyte (Neutrophil myelocyte)
	Bean or kidney-shaped	Neutrophil metagranulocyte (Neutrophil metamyelocyte)
	Curved rod	Neutrophil rabdocyte (Neutrophil staff cell)
	Lobed or segmented	Neutrophil lobocyte (Polymorphonuclear)

* If the granules are scarce, big, and blue, or the cytoplasm contains vacuoles or is bluer than normal, they are toxic neutrophils but are classified otherwise as in the table.

CELL IDENTIFICATION

Having decided on a satisfactory system of nomenclature and classification of the cells and having defined clearly the characteristics of each cell type, the next step was to devise a system of cell identification whereby a technician with a cell under observation which had never been seen before and the name of which was not known could find an illustration and description of the cell and definitely identify it. Borrowing the idea from the system used in qualitative chemical analysis, the accompanying tables 2 to 6 were devised. Use of the tables is based on the answering of the simple questions suggested by the headings.

The first question is, *does the cell contain neutrophil, eosinophil, basophil or azurophil granules?* From the type of granule the cell under consideration contains, look up the further identification in the tables as listed below.

Granules	
Neutrophil	See table 2
Eosinophil	See table 3
Basophil	See table 4
Azurophil	See table 5
No granules	See table 6

If neutrophil, eosinophil or basophil granules are present (tables 2–4), the next question to ask is, *does the nucleus* contain nucleoli? If it does, and the

TABLE 3

EOSINOPHIL GRANULES

NUCLEOLI	NUCLEUS	NAME OF CELL
Present	Round or oval	Eosinophil progranulocyte S (Eosinophil promyelocyte I)
Absent	Round or oval	Eosinophil granulocyte (Eosinophil myelocyte)
	Bean or kidney-shaped	Eosinophil metagranulocyte (Eosinophil metamyelocyte)
	Curved rod	Eosinophil rhabdocyte (Eosinophil staff cell)
	Lobed or segmented	Eosinophil lobocyte (Eosinophil polymorphonuclear)

nucleus is round or oval, the cell is the progranulocyte S (promyelocyte I), corresponding to the type of granule. If nucleoli are absent, the next question to ask is, *what is the shape of the nucleus?* According to the shape of the nucleus, the name of the cell is given in table 3, 4 or 5.

Note that in the tables the column headed "Number" gives the numbers of the cell description and illustrations in the atlas[3], making an index to aid in comparison of the pictures and comments with the cell under consideration. This last column also serves as an index to any specific feature of the cells.

If the cell contains azurophil granules (table 5), the first question is, *is the diameter of the cell significantly greater than 15 micra*, the size of the larger neutrophil lobocytes (polymorphonuclears)? The second question is, *are the gran-*

ules fine and diffusely scattered or are they large and in groups? The third question is, *does the nucleus contain nucleoli?* The fourth question is, *what is the chromatin structure in the nucleus?* The fifth question is, *what is the shape of the nucleus?* The sixth question is, *is the peroxidase stain positive or negative?* The peroxidase stain is only necessary in differentiating the progranulocyte A (promyelocyte II) from the prolymphocyte.

In table 6, the questions to ask in regard to cells having no granules are indicated. First, *is the cytoplasm opaque or transparent?* An opaque cytoplasm looks as if it had been drawn with crayon, whereas a transparent cytoplasm looks as if it had been drawn with water colors. The remaining questions are similar

TABLE 4

BASOPHIL GRANULES

NUCLEOLI	NUCLEUS	NAME OF CELL
Present	Round or oval	Basophil progranulocyte S (Basophil promyelocyte I)
Absent	Round or oval	Basophil granulocyte (Basophil myelocyte)
	Bean or kidney-shaped	Basophil metagranulocyte (Basophil metamyelocyte)
	Curved rod	Basophil rhabdocyte (Basophil staff cell)
	Lobed or segmented	Basophil lobocyte (Basophil polymorphonuclear)

to those already discussed for table 5. It may be worth while to point out, however, that the coarse chromatin structure of the nucleus of the cells of the erythrocyte series and plasmacyte series differs from that in the progranulocyte (promyelocyte) and lymphocyte series in having a very sharp demarcation between the dark-staining basichromatin and the light-staining oxychromatin areas. The latter group of cells have a gradual transition between the dark and light areas of the nucleus. An important point of difference in the chromatin structure between the plasmacyte and the karyocyte (pronormoblast) and prokaryocyte (erythroblast) is that in the plasmacyte the individual masses of basichromatin are much larger than are the individual masses of basichromatin in the cells of the erythrocyte series.

TABLE 5

AZUROPHIL GRANULES

DIAMETER OF CELL IN RELATION TO LOBOCYTE	SIZE OF GRANULES	NUCLEOLI	CHROMATIN STRUCTURE	SHAPE OF NUCLEUS	PEROXIDASE STAIN	NAME OF CELL
Same or smaller	Coarse	Present or absent	Coarse in clumps	Round or oval, sometimes irregular or cloverleaf	Negative	Lymphocyte
Larger	Coarse	Present or absent	Coarse	Round or oval, rarely irregular or cloverleaf	Negative	Prolymphocyte
				Round or oval, rarely horseshoe	Positive	Progranulocyte A (Promyelocyte II)
			Very coarse	Round or oval	Negative	Plasmacyte†
		Present	Fine	Round or oval, rarely horseshoe*	Negative	Lymphoblast or granuloblast
	Fine, diffuse	Present	Fine	Round or oval	Negative	Monoblast
				Horseshoe or irregular	Positive or negative	Promonocyte
		Absent	Coarse clumps and strands	Horseshoe or irregular	Positive or negative	Monocyte
3 times as large	Fine, diffuse	Absent	Coarse clumps and strands	Horseshoe or irregular	Negative	Megalokaryocyte

* Rieder cell.

† Cytoplasm opaque. Plasma cells with azurophil granules are very rare.

TABLE 6

No Granules

All have round or oval nuclei

CYTOPLASM	NUCLEOLI	CHROMATIN STRUCTURE	DIAMETER OF NUCLEUS IN RELATION TO DIAMETER OF CELL	SIZE OF CELL IN RELATION TO NEUTROPHIL LOBOCYTE	PEROXIDASE STAIN	NAME OF CELL
Opaque	Absent	Pycnotic	Less than half	Smaller	Negative	Metakaryocyte*† (Normoblast)
		Coarse	Less than two-thirds	Smaller	Negative	Karyocyte*† (Pronormoblast)
			More than two-thirds	Same or larger	Negative	Prokaryocyte* (Erythroblast)
			Less than half	Usually larger	Negative	Plasmacyte†
	Present	Coarse	Less than half	Usually larger	Negative	Proplasmacyte
		Fine	Less than two-thirds	Usually larger	Negative	Plasmablast
			More than two-thirds	Same or larger	Negative	Karyoblast* (Megaloblast)
Transparent	Present	Fine	More than half	Usually larger	Negative	Lymphoblast, monoblast, granuloblast (myeloblast)
	Present or absent	Coarse	More than half	Larger	Positive	Progranulocyte A (Promyelocyte II)
					Negative	Prolymphocyte
				Same or smaller	Negative	Lymphocyte

* May or may not contain hemoglobin. Other nucleated cells never contain hemoglobin.

† Sometimes two or more nuclei in one cell.

SUMMARY

A simple, logical nomenclature for cells of the blood and marrow is proposed which it is hoped will become standard and permit comparison of differential cell counts made in one laboratory with those made in another. The most important theories of the histogenesis of the blood cells are outlined and the author's present concept of the histogenesis of the cells is presented. Tables of cell identification are given which, it is hoped, will guide the observer of a cell under the microscope to its identity even though such a cell has never been heard of nor seen before.

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* May or may not contain hemoglobin. Other nucleated cells never contain hemoglobin.
† Sometimes two or more nuclei in one cell.

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THE MECHANISM OF THE PRODUCTION OF ACIDOSIS*

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The present wide use of alkalizing therapy justifies a general consideration of the indication for alkalization and its significance.

A general discussion of this subject falls naturally under the following headings:

1. The mechanism of the production of acidosis
2. The forces brought into play by the body in maintaining the normal alkalinity
3. Methods of investigating acidosis
4. The importance of acidosis in certain specified conditions, and the relationship of alkalization to these conditions
5. The opinions of various pharmacologists and therapeutists concerning the efficacy of alkalization.

In the beginning, a clear and concise definition of acidosis is important. In the simplest words, when the bicarbonate of the blood is reduced below normal, acidosis exists. This does not imply that the actual reaction of the blood is changed, but means that the potential alkali of the blood is reduced.

There must be maintained a reserve to neutralize acids which may enter the blood, to stand as a protection against poisoning by such acids. Such reserve exists both in the blood and tissues. Normally, the amount of alkali available permits the blood to combine with from 50 to 70 volumes of CO_2 under definite conditions. The amount present is expressed as "CO_2 combining power," and its estimation is the most widely used means of

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measuring the extent of acidosis. As the alkali reserve is used up by the entrance of lactic or other acids into the blood, the CO_2 combining power decreases.

A simile illustrating the function of the alkali reserve and its influence on well being is that of a business and its capital reserve which is drawn upon to carry the business through times of stress. As long as it is not completely used up, business continues to operate more or less smoothly. The more the reserve is called upon, the closer is the approach to bankruptcy until finally, after all the reserve is depleted, a climax is reached. At this point in order for smooth functioning, the reserve must be bolstered or bankruptcy impends.

Thus it is with alkali reserve in the animal economy. Small changes do not cause symptoms, but they do influence metabolism, just as losses in business, drawing on reserve, bring it closer to the danger point. A business without a proper reserve is not well maintained; a person with even a mild acidosis is not in perfect health.

The causes of this condition are mainly two:

1. Excessive production of acid in the body
2. Defective elimination of acids normally produced
3. A third, but not as frequent cause, may be the ingestion of acids.

EXCESSIVE PRODUCTION OF ACID WITHIN THE BODY

In some metabolic disorders, the normal oxidation of fats is incomplete, viz., does not go on to CO_2 and H_2O, but stops at diacetic or beta hydroxy butyric acids and acetone. This occurs in conditions in which the body cannot completely oxidize carbohydrates—the typical example is diabetes.

Acidosis frequently follows administration of anaesthetics, due to interference with normal metabolism.

It also occurs in conditions in which the power to oxidize may be unimpaired, but the supply of carbohydrate may be deficient.

Under this heading comes:

1. Starvation—abstinence from food
2. Conditions which prevent absorption: pernicious vomiting, cyclic vomiting of childhood, conditions of defective absorption of carbohydrates from the intestinal tract.

In some children symptoms of acidosis frequently arise and are relieved by giving alkali.

DEFECTIVE ELIMINATION FROM THE BODY OF ACIDS NORMALLY FOUND

These fall into two groups:

1. *Defective kidney elimination.* Certain types of renal disease, especially those characterized by marked nitrogen retention.
2. *Retention of CO_2.* Conditions of defective circulation, poor supply of blood to the lung, cause insufficient aeration and decreased elimination of CO_2. In some conditions the state of the lung itself prevents efficient aeration—it may occur in emphysema, pneumonia, pulmonary edema, and morphine poisoning.

Excess ingestion of acids. Hydrochloric acid by mouth; ammonium chloride or calcium chloride.

Acidosis following methyl alcohol poisoning is of this type.

This general discussion, although brief, throws definite light upon the mechanism of the production of acidosis. It is a well known fact that frequently accumulations of organic acids do occur—more frequently than was formerly thought—especially in such conditions as the common cold, la grippe, etc.

THE FORCES BROUGHT INTO PLAY BY THE BODY IN MAINTAINING THE NORMAL ALKALINITY

The body possesses a reserve stock of potential alkali, carbonates and phosphates, plus proteins which prevents changing of the actual reaction of the blood. All this potential alkali must be used up before this actual reaction is changed.

To be more specific, if a certain amount of water required one cc. of normal alkali to alter its reaction, the same amount of normal blood serum would require 300 cc. of the same strength alkali.

The kidneys also take part in the maintenance of the acid base balance and quantitatively excrete 60 to 70 cc. of normal acid a day from the body. The order in which the blood alkalies are used up is first the bicarbonates, then the carbonates and phosphates and tissue, and finally the alkali salts of the bones if the

process is carried far enough (this explains the loss of calcium in acidosis).

Step by step the store of alkali is used up as follows:

1. *Neutralization by buffers.* That is, the bicarbonate, carbonate and phosphate which are the first to come into play and are instantaneous, their action being a simple chemical double decomposition of an acid and a base with the formation of a neutral salt. The buffers of the blood can handle 1000 cc. of normal acid before fatal acidosis occurs.

2. *Respiration.* This is the second mechanism to assert its effect and is also rapid in action, and is chiefly for the purpose of lowering the H_2CO_3 of the blood by added acid. The respiration does this by getting rid of formed H_2CO_3, while permitting 80 per cent of the buffered salts to be neutralized before the pH falls to 7.

Neither of these two body changes act to do anything to restore the body's alkali reserve to normal, that is to say, they do not bolster the depleted store of alkali.

3. *The kidney's excretion of free buffer acids.* Free acids on entering the blood react to form alkali salts, thus causing a primary alkali deficit. In response to this the body excretes some of its phosphate which is transformed in the kidney to the acid form. As each molecule is transformed, it leaves one equivalent of alkali, which remains and reacts with H_2CO_3 to form bicarbonate and builds up alkali reserve. This action therefore is one of replenishment while the neutralization by buffers and release by respiration is merely one of protection. However, it operates much more slowly than the buffers and respiration.

4. *Removal of chlorine ion from the blood.* In some unknown manner, the chloride is removed from the blood leaving the sodium free to neutralize acids.

5. *Combustion of organic acids,* such as oxidation of lactic acid after exercise.

LABORATORY METHODS OF INVESTIGATING ACIDOSIS

1. *CO_2 combining power of the plasma.* This is the most useful test in studying the acid base balance of the blood, and probably one of the most useful of all laboratory tests available. It is done by the gasometric method of Van Slyke, and the results represent the amount of bicarbonate in the blood.

In performing this test it is important that the specimen be handled with a minimum loss of CO_2. The specimen should be collected without stasis, a well oiled syringe being used, with a minimum amount of suction. 5 cc. of blood are required which is transferred into an oxalated tube under oil and mixed with a glass rod, but not shaken. The specimen should be sent to the laboratory as soon as possible but if it is kept on ice in a paraffined tube, may be kept for several days. The result of the test is reported in volumes per cent: 50–70 being normal; 40–50 indicating a moderately severe or "compensated" acidosis.

When the volume per cent declines to as low as 30, definite symptoms of acidosis occur; and when as low as 15, coma is present or is imminent.

In diabetes, the blood sugar is usually 300 mgm. and the urine contains acetone and diacetic acid in large quantities before the CO_2 combining power goes below 50.

The CO_2 of the blood plasma is lower in women and children, hence their greater tendency to acidosis.

2. *Alveolar CO_2.* This is the simplest test for clinical purposes, but is not much used. Air is rebreathed four times from a rubber bag. The sample of air then will have the same CO_2 content as venous blood. The air is bubbled through sodium bicarbonate solution containing an indicator and the solution compared with a standard. Results are expressed in m.m. of mercury or in volumes per cent. 5.5 volumes per cent is normal, this is slightly lower in women and children; 2 per cent indicates coma within 2 hours; 3–4 per cent suggests coma within 3–4 days.

3. *Sellard's test.* In normal individuals 3–5 grams of sodium bicarbonate will cause urine to become alkaline. In acidosis, larger amounts are necessary.

This fact is taken advantage of in estimating the extent of acidosis by Sellard's test, which is reliable and sensitive and has the added advantage that, "the number of doses (of alkali) required to get a neutral urine gives a rough measure of the degree of acidosis while at the same time correcting it"[1].

The technique of the test is to give 5 grams $NaHCO_3$ every 2 hours until the urine becomes alkaline; test before each dose. The specimen is boiled before each test. A tolerance of 20–30 grams indicates moderate acidosis; 40–50 grams, marked acidosis but not enough for symptoms; 75–100 grams, serious clinical symptoms.

If there is marked renal deficiency, the urine might not become alkaline, which renders the test of no value in this condition.

4. *Hydrogen ion concentration (pH) of blood.* The test is not used much because a simple method does not exist and only extreme deviations of CO_2 from the normal can be detected. 7.3–7.4 is the normal limit—7.0–7.8 is the limit compatible with life. Various colorimetric methods are available for measuring pH in this manner. The best known is that of Marriott[2]. Whole oxalated blood is dialyzed against a normal salt solution containing an indicator and the CO_2 is driven off from the latter and the color of the solution compared with suitable standards.

The electrometric method would be the most desirable method, and, with the rapid advancement being made in the technique of making these measurements and more sensitive and stable eletrodes, it is probable that the difficulties at present involved in its use may be eliminated. Nimms et al.[3] have recently reported electrometric measurements of the pH of biologic materials with an accuracy extending to the third decimal place.

The pH of the blood alone however, has only a limited value since it only changes when all of the reserve alkali is exhausted. To be of most significance,

it must be considered in connection with the bicarbonate content, CO_2 combining power. This is apparent when we consider that the following conditions may exist:

1. *Low CO_2 combining power and a normal pH.* This is most often the case and means a reduction of alkali reserve, an acidosis but with still some alkali in reserve—a compensated acidosis.
2. *Low CO_2 combining power and low pH—an uncompensated acidosis.*
3. *High CO_2 combining power, high pH, alkalosis.*
4. *High or normal CO_2 combining power and low pH in acidosis, due to retention of CO_2*

Osgood[4] prefers the titration of determining the alkali reserve. He regards it as one of the simplest and most important of all blood chemical examinations. By this method, the technique of which is given in detail by Osgood, 2 cc. of plasma are decomposed by HCl N/50 and the excess acid titrated to pH 7.4 by N/50 NaOH.

cc. acid − cc. alkali × 22.4 = alkali reserve, expressed in terms of CO_2 combining power. The author finds the method accurate to within 1–1.5 volumes per cent.

In the discussion of alkalization therapy, four questions arise:

1. Does acidosis really occur, as a health factor—when and how?
2. By alkalization, do we mean chemical alkalization in the stomach or do we mean alkalization of the blood?
3. Do the measures employed accomplish this?
4. If they do, is it of therapeutic significance?

The following general discussion deals with clinical, experimental and chemical facts involved in these questions.

THE IMPORTANCE OF ACIDOSIS IN CERTAIN SPECIFIED CONDITIONS AND THE RELATIONSHIP OF ALKALIZATION TO THESE CONDITIONS

The common cold and acidosis. "There is a change in the blood chemistry and consequently there must be a change in the tissues supplied by the blood. There is a decrease in the bicarbonates or reserve bases contained in the blood plasma and the tissues, notably in that of the sodium and calcium salts."

"These findings seem to point the way to the conclusion that a cold is a local manifestation of a systemic disturbance; namely a disturbance of the alkaline balance or reserve—in other words, a mild acidosis, or, perhaps better stated, a lessening of the 'buffer' action of the blood plasma through a decrease in its bicarbonate content. This conclusion is strengthened by treatment in which thorough alkalinization will always abort and cure a cold—a radical statement but nevertheless true, provided the treatment is thorough"[5].

In addition to the two above quotations from this monumental volume of research and publications, some definite facts which have been established in the study of the common cold are listed:

1. The secretions of the nose and throat in the common cold are less alkaline than normal. Such disturbances indicate a mild acidosis.
2. Nasal obstructions, polypi, deviated septum, swollen turbinates, cause a decrease of alkali reserve.
3. Mouth breathing causes a decrease of reserve alkali.
4. In enlarged tonsils, even without nasal obstructions, the reserve alkali of the blood is decreased.
5. All of the symptons of a common cold, from simple coryza to that of la gripple and "flu," have been produced by induction of artificial acidosis by the administration of ammonium chloride, the severity of the symptoms being in proportion to the degree of acidosis produced. The symptoms rapidly subside upon administration of alkali in large doses.

Koehle[6] reports a definite constitutional disturbance, listlessness, drowsiness, and susceptibility to fatigue on slight exertion after producing acidosis by administration of ammonium chloride, 15 grams daily for a week.

Fatigue. Slight accumulations of organic acids occur through muscular fatigue, exertion, and nervous exhaustion—not enough to cause acid reaction, but enough to draw on the acid-base balance.

When the muscles work they produce organic acid substances, commonly referred to as "acid fatigue products." These products being carried in the blood, affect the organs and through them the system as a whole. Normal blood alkali is used up and a mild acidosis results, although the actual reaction of the blood does not change. A prolonged interval of rest is required for the body to adjust this condition by combining these acid substances and removing them. Nature may be aided in this process and protected from this change by a physiological alkali.

A rise in blood lactic acid after severe exertion has been reported[7] and a marked rise after a light exertion was reported by Schenck.

Large quantities of lactic acid were found in the shirts of football players after the Olympic Games in 1929[8].

The lactic acid was found elevated 3 to 4 times the normal after artificially induced high fevers by Fishberg and Bierman[9].

These workers found that the normal bicarbonate of the blood was lowered by the excess lactic acid, even though a great part of it had been eliminated by the sweat.

Alcohol and acidosis. It is an accepted fact that following alcohol, even in relatively small amounts, there is a tendency for the reaction of the blood to shift toward the acid side.

Himwich et al.[10] established that alcohol ingestion caused acidosis by leading to an accumulation of lactic acid in the blood, which disappeared slowly. The

mental and physical depression they attributed, in part at least, to this upset of the acid-base balance.

Some individuals are extremely sensitive and become mildly acidosed with very small quantities of alcohol. The blood was found to be less alkaline and the CO_2 combining power decreased in some of the experimental subjects as long as 48 hours.

Alkalinization and its relationship to salicylate medication. Salicylates should be given with alkali for their best effects, since they themselves tend to cause acidosis.

It is now realized that the intensive use of salicylates, frequently employed, chiefly in the form of aspirin, in the treatment of colds and "flu" results so often in excessive sweating and weakness that this part of the picture has come to be regarded as part of the regular symptoms of the disease.

Thompson and Dragstedt[11] have shown that in dogs the simultaneous use of alkali acts to prevent development of acidosis and gastric irritation as a sequel to salicylate therapy. They proved that calcium also has some protective action on development of salicylism; they conclude, "The ameliorating effect."

Mutch[12] discusses the possibility of loss of minerals from the body following excess intake of aspirin, and concludes that the addition of calcium protects young animals from the harmful effect of aspirin on growing bone.

Acidity may be associated with rheumatism. Acidity with accumulation of uric acid encourages local infection in rheumatism.

Wood[13] states: "It is not improbable that that group of diatheses known as lithaemia, chronic gout, etc., are attended with an increased formation of acid substances. At least, in many of these cases we find the urine highly acid, and the attempt to correct the excessive acidity or diminished alkalinity with the use of alkalies would seem a rational procedure. There are, however, certain salts, especially the citrates, which while themselves practically neutral in reaction, are oxidized in the stomach and appear in the excretions in the form of alkaline carbonates, and these are generally given the preference where it is desired to correct systemic rather than local acidity."

Cushny[14] has pointed out that citrates of the alkalies are largely used in gout, and acute rheumatism, as they render the urine alkaline, and may be of value in both preventing and dissolving some urinary calculi.

Alkalization is good empiric therapy in the treatment of lumbago.

Alkalization of the urine. Alkalization is often a part of the therapy in treatment of pyelitis and urine infections.

The commonest infections occurring in pyelitis are those due to colon bacilli, which produce an acid urine. Changing the reaction of the urine from acid to alkaline and reversing the process several times may frequently overcome this condition. This therapy is commonly used with or without the concurrent use of urinary antiseptics.

In fevers, severe sweating, and diarrhoea, the urine is often concentrated, the consequence of which may be irritation. This condition may be readily overcome by the use of a physiological alkali.

Alkalization as adjunct in the treatment of certain skin conditions. The association of acidosis in conditions such as urticaria or hives, food allergy and certain eczemata, is so well established that in the language of the laity, the conditions are often expressed as "too much acid in the blood." Many physicians in talking to the laymen in terms of the latters' own language, often explain his condition to him in this manner.

It must be conceded that the excretion of an acid perspiration may be an added irritating factor, and is amenable to treatment by alkalization.

Acute infections and fevers. Acidosis is often severe in acute infections and fevers. That acidosis is a part of these conditions is too well recognized now to merit more than mere inclusion in the list and alkalization in some manner is part of the armamentarium of every physician in the treatment of these conditions.

Effervescent alkalies, in addition to protecting the alkalinity of the blood, are ideal means of supplying fluids which are so necessary, act as diuretics, stimulate elimination through the skin, lend themselves admirably to the preparation of grateful refreshing drinks, and make the victims of fevers more comfortable.

Acidosis in diarrhoea and vomiting. By the use of effervescing alkalies the maintenance of the alkalinity of the system may be enhanced by the beneficial effect of carbon dioxide, a quick acting stimulant, which acts in a favorable manner on nausea and upon the digestive tract.

Even low blood pressure has been established as a cause of a lowered alkaline reserve; the manner in which this condition brings it about has been attributed variously to low oxidation activity and to poor circulation.

Cannon[15] reporting on shock, has shown that a CO_2 combining power as low as 40 may accompany a systolic blood pressure of 70–80, and with a systolic pressure of 60–70, it may fall as low as 35 and become progressively lower as the blood pressure falls.

With liver diseases, a lowered alkaline reserve is brought about by the failure of the liver to perform its glycogenolytic function, and with low sugar metabolism, goes acidosis.

THE OPINIONS OF VARIOUS PHARMACOLOGISTS AND THERAPEUTISTS CONCERNING THE EFFICACY OF ALKALIZATION

Meara[1]. Meara refers to the fact that in Sellard's test, acidosis while measured, can at the same time be corrected, which is evidence that alkalies by mouth are an effective means of treating a lowered alkaline reserve.

Meara makes the statement that, "The number of doses (of alkali) required to get a neutral urine gives a rough measure of the degree of acidosis while at the same time correcting it."

Wood[13]. Wood definitely states that alkalies are used to correct reduced alkalinity in the body fluids. This means the blood, lymph, spinal fluid and tissue juices.

Wood concedes that in certain instances there is an increased formation of acid substances and states that, "The attempt to correct the excess acidity or diminished alkalinity with the use of alkalies would seem a rational procedure." He goes further and states that because citrates do not unduly disturb digestion, they are given preference and that they are oxidized and appear in the secretions as alkaline carbonates.

Cushny[14]. Restates the well-known physiology of the body in using alkalies and says, "If the plasma be titrated with an acid, more is required after an alkali has been administered provided the carbonic acid is driven off during titration. After alkali treatment then, the reaction of the blood is unchanged but the alkali available for the neutralization of acid is augmented."

Cushny goes further and deals with the subject quantitatively. He says, "A temporary alkaline reaction lasting two to three hours may often be induced by a single dose of 2–3 grams."

Normally, 20 grams every two hours for eight doses will increase the alkaline reserve by 75 per cent. This begins forty minutes after the first dose and lasts ten hours after the last dose (16).

Is the use of alkali rational. The effect of an acidifying or alkalizing salt on internal acid base balance is equal to the effect of an equivalent amount of the corresponding pure acid or bicarbonate. Twenty grams NH_4Cl or 45 grams of sodium bicarbonate will keep urine of a normal person continuously acid or alkaline as case may be.

As little as 4 grams of bicarbonate or sodium citrate, between meals to a normal person will cause a temporary rise in plasma bicarbonate and a detectable rise in urinary pH.

It has been shown that the rise in blood CO_2 capacity, and hence alkali reserve, caused by a given dose of alkali is equal to the rise which would be expected if the same amount of alkali were added to an amount of fluid equal to 70 per cent of the body weight; or 0.026 gram of $NaHCO_3$ per kilogram body weight will raise the blood CO_2 one volume per cent.

Peters and Van Slyke[2]. Discuss the subject in a manner similar to the above workers and point out how the citrates act to increase the alkali carbonates of the body.

They refer to the work of Henderson who found that as little as 4 grams of bicarbonate between meals produced a detectable rise of urinary pH. Henderson states that the giving of sodium bicarbonate until the urine turns alkaline was a clinical test of the state of acid base equilibrium. The only way it could be this, would be by alkalizing the blood until the excess acid was neutralized.

The advantages of sodium citrate as an alkalizing agent is pointed out.

Himwich et al.[10]. The work of Himwich et al. establishes beyond reasonable doubt that there is a real need for the use of an alkali after ingestion of large quantities of alcohol.

They state 10 cc. of 19 per cent alcohol per kilogram in man is followed by acidosis. This would amount to about one quart or less of wine or its equivalent in any other liquor for a 70 kg. man.

This beneficial effect they believe may be attributed to two things:

1. Direct increase in the alkaline reserve with more rapid neutralization of excess acid.
2. Increase of CO_2 as a biproduct of oxidation of the citrate, with a consequent stimulation of the respiratory center and more rapid oxidation and removal of alcohol.

Wright[17]. Deals with lactic acid and shows definitely how the physiology is carried out in the body.

We know that the lactic acid in the blood is increased after ingestion of alcohol, sometimes to a considerable extent, and here Wright explains the action of alkalies in aiding the system to neutralize it and eliminate it and that, "The reaction of the blood has altered to a much less extent than otherwise would have been the case."

Starling[18]. Concedes the fact that the alkaline reserve can be reduced to the point where its buffer action is lost and then points out that a lowering of the pH of the blood may result upon the addition of acid.

McGuigan[19]. McGuigan states, "A considerable part of the citrates is oxidized in the body to carbon dioxide and water and so renders the blood and urine more alkaline."

RELATIONSHIP OF CALCIUM TO ACIDOSIS

In acidosis, with its associated fatigue and depression, goes blood calcium depletion, as shown by the results of the investigation published in the Annals of the Pickett-Thomson Research Laboratory on the common cold. The value of calcium also in modifying the action of salicylates has been referred to. Solis-Cohen touches on the value of using an alkalizer which does not increase by repeated administration, the excretion of lime and thus does not alter the calcium content of the blood.

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NEWS AND NOTICES

Tumor Registry Loan Sets may now be obtained on application to the Secretary.

The rental charge for such sets is $3.00 for one week, an additional charge of $2.00 being made for each additional week. A breakage deposit of $20 is required which is returned when the sets have been returned intact.

Sets will be shipped express collect and are to be returned express prepaid.

A meeting of the clinical pathologists of New Jersey was held in Newark October third at the Academy of Medicine of Northern New Jersey for the purpose of forming a New Jersey Society of Clinical Pathologists.

The following officers were elected:

President: Asher Yaguda, M.D., Newark, N. J.

Vice President: Robert A. Kilduffe, M.D., Atlantic City, N. J.

Secretary: A. J. Casselman, M.D., Camden, N. J.

Treasurer: A. Casilli, M.D., Elizabeth, N. J.

The Executive Committee is composed of: Dr. Robert A. Kilduffe, Chairman; Dr. Asher Yaguda, Dr. A. J. Casselman, Dr. A. Casilli, Dr. H. A. Martland, Dr. J. W. Gray, and Dr. M. J. Fein.

For the first time in its history a Pathological Section was formed in the New York State Medical Society. The following officers and committees were appointed:

Dr. James Ewing, Chairman.

Dr. N. Chandler Foot, Vice-chairman.

Dr. M. J. Fein, Secretary (50 Greene Ave., Brooklyn, N. Y.).

Committee on Scientific Exhibits: Dr. Ward J. MacNeal, Chairman, Drs. Klemperer, Stout, Stewart, and Sondern.

Committee on Manuscripts: Dr. N. C. Foot, Chairman, Drs. Fein, Marten, and Graef.

The Society for Investigative Dermatology

A new society, the Society for Investigative Dermatology was founded on June 10, 1937, and will publish a new journal, the Journal of Investigative Dermatology.

The officers and board of directors are:

Dr. George M. MacKee, New York, President

Dr. Joseph V. Klauder, Philadelphia, Vice-president

Dr. S. W. Becker, Chicago, Secretary

Dr. J. Gardner Hopkins, New York, Treasurer
Dr. S. Pollitzer, New York
Dr. John H. Stokes, Philadelphia
Dr. Hamilton Montgomery, Rochester, Minn.
Dr. S. M. Peck, New York
Dr. Marion B. Sulzberger, New York

The objectives of the new society and the journal include the effort to assemble under one cover all the numerous types of investigative work dealing with the skin, its functions and reactions, both physiologic and pathologic, a field not hitherto covered by other societies and journals. The proposed first annual meeting and scientific program of the society will be held in the Spring of 1938. All papers on investigations dealing with the skin, or its actions and reactions, or on investigative syphilology may be submitted to

The Journal for Investigative Dermatology
Dr. Marion B. Sulzberger, Editor
962 Park Avenue
New York City

It is expected that the first issue of the Journal will appear in January, 1938.

Have You Met "Dr. Benjamin?"

If "Dr. Benjamin" should happen to present himself as an associate of Dr. Simmons in the Department of Pathology at Northwestern University with a hard-luck story of one kind or another—you are meeting an impostor. This gentleman may want you to endorse a check, borrow money, or get the names of other pathologists in your vicinity. Before you do any of these things for him, see the February 13, 1937 issue of the Journal of The American Medical Association, page 567 or the issue of November 6, 1937, page 1552.

Then throw him out (if you are big enough), or call the police if you have already had your quota of exercise for the day.

This man is of average size, about 40 years old, with hair tinged with gray. His face is round with small, dark, slightly prominent eyes and his neck is short and thick. He is familiar with medical terminology, has a fair knowledge of laboratory methods, and may talk quite familiarly of pathologists he knows.

Be on your guard against this impostor.

The New Jersey Society of Clinical Pathologists Presents a Plan

For the purpose of assisting in the coordination of the facilities of the state in the campaign against syphilis, the Executive Committee of this newly formed society formulated a tentative plan for those phases of the campaign properly falling within the province of the clinical pathologists. This plan is to be presented for discussion to the President of the State Medical Society, The Venereal Disease Control Committee of the State Medical Society, the Welfare Committee of the State Medical Society and, after a definite plan has been agreed upon, to the State Department of Health.

The proposed plan in full follows:

The ultimate success or failure of the campaign against syphilis inaugurated by Surgeon-General Parran of The United States Public Health Service will depend upon two primary factors of obvious and essential importance: First, the mobilization of effective measures for the recognition of the existence of the disease; and, second, the mobilization of effective methods for its treatment and control.

It is thus apparent that to carry the campaign to a successful conclusion will demand the active cooperation not only of physicians in general, but of clinical pathologists in particular.

It is obvious that the solution of the problem begins with the diagnosis of the disease and it is unnecessary to dilate upon the difficulties with which this may at times be surrounded. Nor is it necessary to discuss at length the care which must be taken to avoid an erroneous diagnosis of syphilis, on the one hand, or the failure to detect a potential focus of infection on the other. The importance of avoidance of such errors—in so far as it is humanly possible to avoid them—is apparent.

While the clinical study of syphilis is essential and can never be neglected without disaster, laboratory methods, such as the dark-field examination and the various serological procedures, are of equal and, in some measure, of paramount importance. For, as is well recognized, under many circumstances serological evidence may be regarded as the most delicate and constant *single* symptom of the disease.

Because of these facts, the laboratory stands in the forefront of the campaign and, by the same token, the clinical pathologist occupies a crucial position and becomes, in fact, the key-man of the situation.

It is readily apparent that, just as it is impossible for either the State or Federal Government to devise or expand an adequate personnel for adequate therapeutics, so present State or Municipal laboratory facilities are entirely inadequate to take care of the volume of work incident to the conduct of the campaign; nor can they be readily expanded to the necessary degree without much delay and prohibitive expense.

This difficulty is solved by the employment of the present medical profession on a limited fee basis for the treatment of syphilis.

Diagnostic facilities can be secured in the same manner by utilizing to the fullest extent the services of the clinical pathologists of the community or state.

Recognizing this fact, and appreciating that the clinical pathologists and their laboratories form an already existing, strategically located network throughout the country, the United States Public Health Service has said that "It is not deemed feasible or advisable to restrict the performance of blood serologic tests to a central state laboratory," nor, by the same token is it advisable to establish branch laboratories for the purpose.

Surgeon-General Parran is definite in his desire that, in the necessary utili-

zation of laboratory facilities the fullest possible use be made of existing private and hospital laboratories, even to the point of stimulating by subsidy the establishment of private laboratories by clinical pathologists where none are now available and, by the withdrawal of state laboratory facilities, if necessary, in order to make the volume of work going to such private laboratories sufficient to secure an adequate return.

Any other procedure must inevitably jeopardize the existence of the clinical pathologist and discourage entrance into this specialized field of the practice of medicine. And, should this happen, the deterioration of medicine as a whole will have begun and will inevitably continue. For there must be clinical pathologists—physicians trained in the study of the causes and mechanisms of disease and trained in the recognition and evaluation of their manifestations and aftermath—there must be clinical pathologists to enable the study, elucidation, and control of disease, to assist the physician in his diagnostic problems, and in the newer and highly developed biologic methods of treatment; and to supervise the activities of clinical laboratories, whether under Government, state, municipal, hospital or private auspices.

These are the facts. In order to assist in the solution of these problems and to enable the efficient utilization of the laboratory facilities now existent in this State, the New Jersey Society of Clinical Pathologists presents, for consideration and discussion, a working plan for their participation in the syphilis campaign.

This plan embodies and is based upon several premises which may be thus outlined:

1. That, by virtue of the distribution of pathologists and their laboratories throughout the State there now exists sufficient laboratory facilities to render unnecessary expansion of State or Municipal laboratories for the specific purposes of the syphilis campaign.

2. That such money as is to be allotted for the utilization of laboratory procedures may be most wisely and economically expended in the utilization of already existing private and hospital laboratories which are now equipped for this work.

3. That a pro-rata allotment of such money on a cost-plus basis allowing a reasonable—though slight—profit will not only enable a satisfactory and efficient laboratory coverage, so to speak, but will, in so doing, conserve the existence of clinical pathologists as an essential part of the practice of medicine.

It is of primary importance that, as the utilization of laboratory procedures and their intelligent and safe interpretation in the study of syphilis constitutes, as is obvious, a phase of the practice of medicine, any arrangement involving the utilization of the services of a pathologist working in an institution must apply solely and directly to the pathologist and not to the institution.

It should be further emphasized and reiterated that serologic procedures, which must be safeguarded by their relegation to the hands of those fully trained in their complexities, fully cognizant of their inherent vagaries, and

thoroughly conversant with their clinical interpretation and significance—it must be emphasized and reiterated that such procedures must not be blindly relied upon as the sole basis of the diagnosis of syphilis, but used rather as a means for selecting those cases in which further studies may profitably be made before the grave and practically irreversible diagnosis of syphilis can justly be made.

With these aims in view the New Jersey Society of Clinical Pathologists presents in this report the following detailed and specific plan for participation in the Syphilis Campaign:

1. The state to be divided into units or districts comprising one or more local units of government, such as city, county, or more than one county, depending upon population and availability of clinical pathologists.

2. Clinical pathologists who may participate in this campaign must be those approved as competent in this field of medical practice by the American Board of Pathology or by the State Department of Health. Certification constitutes such approval.

3. In districts where there is no governmental laboratory doing serology, the physicians in that district should be notified by letter by the State Department of Health or the Venereal Disease Control Bureau to send their bloods and spinal fluids for serologic diagnosis of syphilis to designated clinical pathologists in that district.

4. In communities or districts where governmental agencies doing serology already exist, specimens of blood and spinal fluid collected by physicians in the campaign against syphilis should be handled in one of two ways:

a) The district redivided into smaller divisions and clinical pathologists designated for each division and collectors instructed to deliver all specimens in that district to the designated clinical pathologist after the estimated load of the governmental laboratory has been apportioned to it.

b) All specimens to be delivered to the governmental laboratory, which, in turn, redirects all specimens in excess of its normal load to designated clinical pathologists in the district.

Where the clinical pathologist designated does any serologic examination under this agreement in a hospital laboratory, it must be specifically stated in the agreement that the arrangement is with the pathologist and not with the institution.

5. Where large numbers of specimens may accrue from one given source, such as a large industrial plant, a large penal institution, etc., the entire load of such institution may be apportioned to the clinical pathologists in the district in which the institution is situated.

6. Darkfield examinations of primary lesions may be handled in a similar manner, providing the facilities already set up by the Venereal Disease Control Bureau should at any time prove inadequate.

7. All of these foregoing items are tentative and may be amended or adjusted to meet conditions found by the State Department of Health or the Venereal Disease Control Bureau.

Fee Schedule

For the purpose of this campaign the following tentative fee schedule is recommended.

1. The fee shall be two dollars per serologic examination where the number of examinations per month does not exceed fifty.

2. The fee shall be one dollar per examination where the number of examinations per month does not exceed two hundred.

3. The fee shall be seventy-five cents per examination where the number of examinations per month exceeds two hundred.

Fee schedule for Darkfield examinations: Five dollars per darkfield examination for Spirocheta pallida.

Correspondence

To the Editor:

In the September issue of the American Journal of Clinical Pathology under the title "Caveat Emptor" you discussed at some length a test for syphilis which you did not designate by name. Some of your remarks have led me to believe that you were referring to the Laughlen Test which was published in the Canadian Medical Association Journal of August 1935.

You prefaced your discussion with a plea for accuracy and I am willing to believe that you intended your statements to be accurate. It is because of a number of very apparent and important inaccuracies in your statements that I am prompted to write this letter trusting that you will not fail to make the necessary corrections.

You stated that the results of only 400 blood samples and 20 spinal fluid samples formed the basis of the original publication of this test. It was clearly stated in that publication that 400 unknown blood samples, 20 unknown spinal fluid samples, several hundred known positive blood samples, 118 unknown blood samples from cases under treatment and work done in other independent laboratories formed the basis for the publication. In other words, while the series was admittedly small, you have given credit for less than half the cases actually reported.

You also stated that the method had not been sufficiently tried and that only the one published report had been made. It is quite inaccurate to state that the test has not been thoroughly checked. For 2 years the materials have been placed at the disposal of clinical laboratories in Canada and the U. S. A. for trial purposes and extensive records are now available. At least 2 other independent reports on the test have been published and both authors obtained substantially the same percentages of agreements between various methods as were reported in the original article.

I also take exception to your stand that hospital internes should not perform tests for syphilis. The Laughlen Test presents no greater technical difficulties than the matching of blood in selecting a blood donor and the pro-

cedure is much the same in both. Many institutions entrust the blood matching to internes. It is true that syphilis tests done by them should be checked by the clinical pathologist and it is equally true that blood matching should be checked in the same way. A mistake in selecting a proper donor can result in more serious consequences than an error in reading a test for syphilis. The Laughlen test is an ideal one for emergencies when only an interne is available.

Neglect to perform a test for syphilis is in some institutions an offense and rightly so, as a case I encountered recently goes to prove. A donor was tested in my laboratory by this method and found positive: when questioned he admitted that he had received treatment and had given his blood as a donor twice in another institution. A test by an interne is better than none at all, especially if under such circumstances it is used to exclude the existence of syphilis. The Laughlen reagent for this purpose can be kept ready for use at a moment's notice and thus removes any delay or excuse for omitting a blood test.

You took objection to one phrase in the instruction sheet which states that one using the test must assume some responsibility for using only good active reagent: your contention being that the operator assumes full responsibility in every case.

You are right only in those cases where the operator prepares the antigen, the reagents and all the materials. In this case the antigen, and the stock reagent are prepared in a central laboratory and the only responsibility of the technician, in respect to the reagent, is to add saline as instructed and to discard any of this active reagent that remains unused as soon as it becomes overactive. The greater responsibility rests with the manufacturers, for without good reagents technicians cannot carry out proper tests no matter how careful they may be.

I share with the manufacturers in this greater responsibility since I check each new lot of reagent which they prepare.

You took objection to the statement: "the Laughlen Test could be done by a medical practitioner."

Perhaps some practitioners cannot be trusted to carry out a simple laboratory procedure. If any doctor wishes to carry out tests for syphilis the Laughlen Test is the simplest and safest one for him to use since he can secure the reagent ready for use and about all he has to do is place a quantity of reagent with an equal quantity of blood serum. Such a test is safe in the hands of a careful painstaking doctor who makes his diagnoses from clinical evidence supported by serological examinations of the blood.

In concluding, you ask a question which I do not hesitate to answer. No one should be expected to accept a diagnosis of syphilis on himself from the positive result of any blood test. The diagnosis is properly made from the clinical evidence supported by laboratory findings.

GEORGE F. LAUGHLEN
495 Broadview Avenue,
Toronto, Canada.

(In publishing the Editorial in question the Editor had no intention of initiating a controversy, nor in adding these comments is there any desire to enter upon one. Nevertheless, in view of the importance of the principle in question, some further comment may be ventured.

The point at issue is not whether the Laughlen test is a good test for serologic evidence of syphilis but whether this test—or any other, for that matter—can safely be broadcast as suitable for indiscriminate use.

It may be that Dr. Laughlen has devised what some clinicians have long sought—a "fool-proof" serological procedure the results of which are infallible in *all* hands under *any* circumstances. But this assumption seems open to some legitimate doubt and requires confirmation.

A new test for syphilis must win its spurs by extensive and critical comparison in many hands in terms of thousands, and the results of such studies must not be buried in personal archives but, by publication, made freely available for critical analysis.

As stated in the Editorial, there was on record when it was written, but one report other than Dr. Laughlen's. If others have been made incorporating the experiences of the laboratories in question, they have not yet come to the Editor's attention nor has Dr. Laughlen furnished copies or reprints of the data to which he refers.

The Editor is still of the belief that, in view of the inherent and ineradicable complexities of serologic procedures applied to syphilis, something more is required for their safe utilization than mere access to the necessary apparatus and reagents. One may readily secure the instruments necessary for performing a hysterectomy—but the requisite surgical skill is not thereby so easily acquired. Nor is the difficulty removed in toto by the fact that the reagents are supplied from a central laboratory—any more than surgical skill is ipso facto conferred by the fact that one's set of instruments comes from a deluxe manufacturer.

Moreover, the reagents for the Laughlen test as received from the manufacturer still require manipulation by the recipient. It is necessary to avoid hypersensitivity of the antigen, to recognize the various factors which may produce anomalous reactions—some of which are briefly referred to in the leaflet advertising the product—and, which is not referred to in the leaflet, quite essential to learn to distinguish between doubtful and negative reactions.

Dr. Laughlen says that "the only responsibility of the technician in respect to the reagent is to add saline as instructed and to discard any of this active reagent unused as soon as it becomes overactive."

But is not *some* serologic training and experience at least useful in detecting *when* an antigen has become hypersensitive? Is it really true that all one has to do "is place a quantity of reagent with an equal quantity of blood serum?" No precautions, no other skill or knowledge than this necessary to safeguard the procedure—and the patient?

As one with some experience in serology the Editor still believes that it is necessary for those engaged in the conduct of serologic procedures in syphilis to accept—and be fully prepared to accept—not "some" but complete responsi-

bility for their results. It is still necessary, therefore, for them to acquire a definite degree of thorough understanding of the principles and technical minutia involved. That internes, physicians, or office technicians in general are uniformly so informed and generally so trained can hardly be without question. It can be maintained without fear of successful contradiction that to remove the safeguards which should surround the performance of all tests for syphilis is to invite inevitable disaster, particularly for the patient.

It may also be maintained, without much fear of question, that *the unrestricted dissemination of any serologic procedure applicable to syphilis in the hands of those untrained in their basic principles and unskilled in their use cannot fail to be disastrous in no small proportion of cases.*

This is true whether it be a complement fixation test, or a flocculation test such as Laughlen's or the Ide test which, in principle, it closely resembles.

It goes without saying, of course, that not only blood grouping and a direct compatibility test, as well as some test for serologic evidence of syphilis, should precede a transfusion. It also goes without saying that *those who apply such tests must first have knowledge of their basic principles, acquire skill in their performance and, especially, by experience learn the pitfalls to be avoided.*

It may be that there are hospitals in which such important matters as blood matching and determination of the suitability of donors are left to internes. They are not to be commended without reserve for that. When tests for syphilis are neglected as a sine qua non in the selection of a donor, an example of what may follow is cited by Dr. Laughlen. But if the Laughlen test is just the thing for an interne to do, why should it be checked by the pathologist as Dr. Laughlen says it should? And if it should be checked under such circumstances, who should check it when done promiscuously by any one? If it is so simple and fool-proof and invariably correct and safe and incapable of error no matter who performs it or how—why need it be checked at all?

Dr. Laughlen purports to answer the question with which the Editorial concluded—and no one will cavil at his reply. But the question was based upon the instruction leaflet which conveys the impression that "the test's the thing"—it's either positive or negative, you have it or you don't.

The excerpt following, from an address made by Dr. James Gregory, Professor of Medicine in the University of Edinburgh (1790–1821) seems pertinent: "I do not know, nor can I conceive of any human contrivance that can more effectually and irresistibly oblige the physician to study carefully the case of his patient; to study every symptom and change of symptom; to exert himself to the utmost for his patient's relief, and at the same time be as careful as possible in the remedies he employs; than to find himself under the necessity of giving a minute account of everything he has done in a very public manner and before a number of competent judges."

Under these circumstances it is not unreasonable to suppose that there might be some embarrassment attached to a diagnosis of syphilis largely based upon a ten minute test with two cents' worth of reagents, the technic of which had been acquired in a few minutes' time from a small sales leaflet.

R. A. K.)

BOOK REVIEWS

Clinical Laboratory Diagnosis. By SAMUEL A. LEVINSON, M.D., Director of Laboratories, Research and Education Hospitals, Chicago; and ROBERT P. MACFATE, Ch.E., Assistant Director of Laboratories, Research and Educational Hospitals, Chicago. Cloth, 877 pp.; 144 illustrations and 14 plates, $9.50. Lea and Febiger, Phila., Pa.

This is a most comprehensive text on clinical laboratory procedures and their interpretation and will undoubtedly take its place as a standard reference text for the laboratory, the physician, and the pathologist.

The methods presented are mainly those in use in the laboratories of the Research and Educational Hospitals, and, as may be expected from the experience and reputation of the authors in this field, represent the advances made in clinical laboratory procedure.

Whenever necessary for the proper correlation of clinical and laboratory findings a brief review is given of the anatomy, physiology, and biochemistry pertinent to the subject. This feature of the book, as well as the short reviews of outstanding diseases, will be of especial value to the physician in search of specific information applicable to a specific problem, as also will be the chapter on skin tests and other biological examinations.

The book differs somewhat from other standard texts on this subject in the inclusion of a special chapter on laboratory methods in pediatric procedures and a rather comprehensive chapter on legal medicine and toxicology. A special appendix contains a detailed outline of a course in clinical pathology as given by the authors.

This book will find its way to the laboratory library as well as that of the physician as a valuable and comprehensive reference text. It may be cordially recommended.

Dr. Bodo Otto And The Medical Background Of The American Revolution. By JAMES E. GIBSON. Cloth, 345 pp., 7 plates, $4.00. Charles C. Thomas, Springfield, Ill.

Dr. Bodo Otto was of Saxon ancestry and for twelve years was Chief Surgeon for the Fortress of Kalkberg. In 1755 Dr. Otto with his family emigrated to America and located in Philadelphia.

This book is the story of his subsequent career, not only as a physician in colonial days, but also as a Surgeon in the Continental Army. This alone would suffice to make an interesting story, but the book—as implied in the title—is much more than that.

It is an account, well written and well documented, of the physician and the medical practice of early Colonial America, and a tale of absorbing interest of the trials, the tribulations and the occasional triumphs of the military surgeons of the Revolutionary armies.

Starting with a picture of medical practice in Germany in the days of 1650, the book then turns to medical practice in Philadelphia in colonial times; to the gradual establishment of a medical service to Washington's army and its development during the course of the Revolution. The regime of the continental medical department under Morgan, filled with acrimonious complaints and recriminations culminating in his dismissal as Director; the medical department at Valley Forge; the famous quarrel between Rush, Morgan, and Shippen; the court-martial of Dr. Shippen and its aftermath, are all recounted in a most interesting manner.

Finally, there comes the post-war period when Dr. Otto settled in Reading, Pennsylvania, where he died and was buried June 15, 1787.

This book is replete with interest. It should appeal to all who are interested in medical history, especially to those interested in early American and Revolutionary history, and in general to the reading public.

Once begun it will not readily be laid aside until the last page has been turned.

Atlas of Hematology. By EDWIN E. OSGOOD, A.M., M.D., Assistant Professor of Medicine and Head of Experimental Medicine, University of Oregon Medical School, and CLARICE M. ASHWORTH, Medical Illustrator, University of Oregon Medical School. Cloth 255 pp., 325 colored plates, $10.00. J. W. Stacey Inc., San Francisco.

Dr. Osgood's reputation as a hematologist, teacher, and investigator in the field of hematology suffices to ensure for this book a place among the outstanding texts upon hematology.

Even were this not so, the book itself would command attention, for in many respects it is unique.

As stated in the preface, "It's major theses are, first, that accurate diagnosis is prerequisite to good therapy, second, that a systematic hematologic study will aid materially in the diagnosis of almost any disease. . . . Its aim is to show the physician and student how to plan and interpret this examination and the technician how to perform the laboratory phase of it.

Any one called upon to make cytological blood studies will readily admit the difficulty not infrequently encountered in the identification of particular cells. One of the purposes of this atlas is to minimize this difficulty so that, once the salient characteristics of the cell have been noted, by means of the tables, descriptions, and illustrations it may be identified "even though the observer has never before seen nor heard of it."

It will readily be seen that this is an ambitious project and in this respect

the atlas is indeed unique. It is obvious, also, that with this purpose in view the major emphasis of the atlas must be, as it is, on morphology.

The book naturally begins with a discussion of the histogenesis and nomenclature of blood cells. The author's concept of the histogenesis of the blood is illustrated in the frontispiece and discussed in the opening chapter. Dr. Osgood's position is midway between the monophyletists and the polyphyletists.

No one will dispute the confusion of hematologic nomenclature and it is to this section of the book that the comments of hematologists may be largely directed. Here the author has introduced—on logical and clearly stated grounds—a new terminology for the granulocyte (myeloid) and erythrocyte series. In every case, however, both the new and the most nearly equivalent older terminology are given in the text to avoid confusing the reader. A table of nomenclature presents clearly the new terms suggested, the preferred older term, and also the many other names which have been applied to the same cell.

Identification of cells by means of this book depends primarily upon determination from a properly prepared and stained smear of the following characteristics: presence or absence of granules and their nature; presence or absence of nucleoli; and shape of nucleus. By reference to appropriate tables, certain other characteristics to be sought for are developed and the reader is finally referred to the colored plates illustrating the cells of the series in question from comparison with which the cells seen may be identified.

The chapters describing the blood cells (I–X) are followed by seven chapters in which the hematological aspects of disease in general and diseases of the blood in particular are excellently and clearly discussed. This section should prove of great interest to the physician and clinical pathologist. There is finally an appendix of methods, an extensive list of references, both general and by chapters, and a full and comprehensive index.

It is readily apparent that the value of such a book as this is largely determined, in the last analysis, by the character of its illustrations. The author, the illustrator, and the publisher alike deserve the highest commendation for the excellence of the plates and the exceptional manner in which they have been reproduced.

This reviewer has seldom seen colored reproductions of stained blood cells which so closely approximate the actual picture seen under the microscope.

It may be predicted with confidence that this atlas for many years will serve a useful purpose, not only as a medium for the instruction of the student and the student technician but as a reference text for the physician, clinical pathologist, and hematologist.

No matter how extensive one's hematologic book shelf may be, this volume can be added to it with profit.

Human Pathology. By HOWARD T. KARSNER, M.D., Professor of Pathology, Western Reserve University. With an introduction by Simon Flexner, M.D. Ed. 4, Cloth, 1013 pp., 443 illustrations, and 18 colored plates, $10.00. J. B. Lippincott, Phila., Pa.

Time was when pathology was regarded as concerned mainly with morphology and, as such, regarded as having but a more or less academic relation to the practice of medicine. Today, as Dr. Karsner aptly remarks in his Preface, "clinical medicine is applied pathology."

His book is written from this viewpoint and those who are familiar with it will not be surprised, therefore, that it has reached a fourth edition.

The book adheres to the conventional division into general and special pathology which extensive teaching experience has shown to best serve the purpose in presenting pathology as an introduction to and basis for the clinical branches.

The first twelve chapters (330 pages) are concerned, therefore, with the presentation and discussion of the fundamental phenomena comprising the mechanism of disease, its causes, processes and effects.

The remaining ten chapters are given to the presentation of systemic pathology, the discussion of specific diseases and their specific effect upon the system in general and upon specific systems and organs in particular.

The text in general evidences an ample and comprehensive experience, both in the practice and teaching of the subject.

Professor Karsner not only knows his subject but possesses the fortunate ability to impart his knowledge. The style is clear and easy; the subjects well covered, the modern advances and current literature well and fairly presented.

The illustrations are not only numerous but well chosen and well reproduced.

A list of pertinent references is appended to each chapter and there is a comprehensive index.

This book can be recommended, not only to the student, but also to the pathologist and clinician as a valuable reference text.

Trauma and Disease. By LEOPOLD BRAHDY, M.D., Physician in Charge of Industrial Diseases and Accidents in the office of the Corporation Counsel of the City of New York, and SAMUEL KAHN, M.D., Medical Examiner in the Bureau of Workmen's Compensation of the Department of Labor, State of New York. Cloth, 613 pp.; 9 figures, $7.50. Lea and Febiger, Phila., Pa.

Among the many problems which physicians in general, and often pathologists in particular, are called upon to attempt to solve is the determination of the possible and probable relation between disease and a preceding single trauma.

It is true beyond question that, in view of the present compensation acts and the prevalence of "accident insurance," the tendency of the layman is to attribute to trauma an undue and often unwarranted importance. It is also true,

as pointed out by the authors in their preface, that physicians are sometimes prone to minimize the significance of injury, on the one hand, or, on the other to attribute to it too great importance, in each instance very often on a doubtful and purely theoretical basis.

As Brahdy and Leopold also point out, at least some of the marked diversity of medical opinion in such cases arises from the lack of complete and accurate data.

The purpose of this book is to "present the accumulated knowledge concerning the relationship of a single trauma to disease, to indicate the limitations of this knowledge, and to develop the underlying principles on which, in any given case, the medical opinion should be based."

The value of such an authoritative survey and discussion cannot be overemphasized. As the book is specifically concerned with the effect of a *single* trauma—physical or psychic—in producing or influencing disease, its importance and value to the industrial surgeon, to the Compensation Referee, to the pathologist, the physician at large—to all who may be concerned with this problem or find themselves involved in its discussion, is obvious and paramount.

Very wisely—a feature which greatly enhances the value of the book—the authors have based their discussion, not on court decisions or the opinions (not always unbiased) of medico-legal experts, but upon authorities in individual fields of medicine. The twenty-four contributors are thus men of varied and extensive experience to whose opinions great weight must be given.

This book can be highly recommended as an important and authoritative contribution to a very controversial subject which, without doubt, deserves an enthusiastic reception.

Clinical Allergy. By LOUIS TUFT, M.D., Chief of Clinic of Allergy and Applied Immunology, Temple University Hospital, Philadelphia, with an INTRODUCTION by JOHN A. KOLMER, M.D., Professor of Medicine, Temple University. Cloth, 711 pp.; 76 figures, $8.00. W. B. Saunders Company, Philadelphia, Pa.

Allergy and its manifestations are attracting much attention at the present time, all the more so because the subject is complex and its manifestations not easily interpreted.

Not only is the mechanism of the condition itself far from understood, but, as a more or less direct consequence, the methods for the management and control of allergic manifestations is also still in process of evolution.

Dr. Tuft's book, therefore, should be welcomed by students of this problem and particularly by the physician confronted with its practical aspects, for it is not only comprehensive but eminently practical in outlook.

In this book, Dr. Tuft has summarized the present knowledge of the clinical manifestations, diagnosis, and treatment of allergy in an excellent and practical way.

The book is divided into four main sections: I The fundamental principles of allergy and anaphylaxis as well as those governing diagnosis and treatment; II The basic etiologic types responsible for and important in most allergic conditions; III Clinical manifestations of allergy, exclusive of those involving the skin; and IV Allergic dermatoses and allergy in relation to the specialties. In this section a special chapter is devoted to allergy in children.

An appendix presents an excellent discussion of laboratory methods in anaphylaxis, laboratory methods in allergy, directions for asthmatic and hay fever patients, lists of allergens and their sources, allergic diets and recipes and other information of practical value.

There is an extensive bibliography and a comprehensive index.

The style is easy and the discussions throughout the book bear the stamp of extensive practical experience and familiarity with the literature.

The author has adapted for the presentation of illustrative cases, methods and the like, the table arrangement used by Stokes.

This volume may be looked upon as a very useful text of value to physician and student alike.

Lobar Pneumonia And Serum Therapy. FREDERICK T. LORD, M.D., Clinical Professor of Medicine, Emeritus, Harvard Medical School, Member of The Massachusetts Advisory Committee on Pneumonia, and RODERICK HEFFRON, M.D., Field Director, Pneumonia Study and Service, Massachusetts Department of Health. Cloth, 91 pp.; 10 figures, $1.00. The Commonwealth Fund, New York.

This is the first of three monographs in which will be reported and analyzed the results of the study of pneumonia begun in Massachusetts in 1931.

The present volume presents the present conception of certain aspects of lobar pneumonia and the methods of application and the results of serum therapy.

In Massachusetts suitable treatment with potent serum effected a definite decrease in the death rate of Type I and II pneumonia. The study has likewise shown that serum can be administered advantageously in the home by the practitioner at large, providing the entire situation is thoroughly understood.

The main purpose of the book is to present the subject of serum therapy in pneumonia in such a manner as to encourage the use of this agent in a proper manner and in such cases as are best suited to the use of this method of treatment.

The manner in which the subject is discussed can be seen from the table of contents: The Application of Specific Therapy to Lobar Pneumonia; Definition and Etiology; Factors Influencing Recovery; Clinical Diagnosis and Selection of Cases for Treatment; Recognition of Type of Pneumococcus Infection; Antipneumococcic Serum; Precautions Prior to Serum Administration; Administration of Serum and Dosage; Serum Reactions and Their Treatment; Results of Serum Treatment.

Although the book is small and compact it contains a wealth of practical information presented in an eminently practical manner and is thus of great value, not only to the practitioner but to all who are interested in pneumonia and its management.

Textbook of Medical Bacteriology. By R. W. FAIRBROTHER, M.D., Lecturer in Bacteriology, University of Manchester. Cloth, 437 pp., 12 figures, 4 colored plates, $4.50. C. V. Mosby Co., St. Louis, Mo.

As stated in the Preface—and as is apparent from the text—this English volume is intended primarily for the medical student and has for its aim the discussion of "bacteria as agents of disease in man and the application of bacteriological methods of the prevent, diagnosis, and treatment of disease."

The book is divided into three main sections: I General Bacteriology, II Systematic Bacteriology, and III General Technique.

The first two sections present a well written and easy flowing narrative of bacteria and their relation to disease and of the methods applicable to their study, without, however, discussing technique except in a very minor way. The last section, devoted to technique is very brief—too brief to be of interest to the laboratory worker and too general to be of great assistance to those seeking information on methods.

The book is well planned to serve the end in view and should prove useful to the medical and dental student, the nurse, and to the physician desirous of surveying rapidly the modern concepts of bacteriology and its relation to disease.

Diseases of The Blood and Atlas of Hematology. With Clinical and Hematologic Descriptions of the Blood Diseases, Including a Section on Technic and Terminology. By ROY R. KRACKE, M.D., Professor of Bacteriology, Pathology and Laboratory Diagnosis, Emory University School of Medicine, and HORTENSE ELTON GARVER, M.S., Instructor In Laboratory Diagnosis, Emory University School of Medicine. Cloth, 532 pp., 44 colored plates and 17 figures, $15.00. J. B. Lippincott Co., Philadelphia, Pa.

Dr. Kracke's reputation as a hematologist is outstanding and his demonstration of the relation of drugs carrying the benzol ring to the production of malignant neutropenia is an instance of his skill in the field of hematologic research.

It is to be expected, therefore, that this book would be an outstanding production and this expectation is amply fulfilled.

The volume is divided into eight main sections: I. Hematologic Terminology (15 pp.); II. The Development and Morphology of Blood Cells (56 pp.); III. Leukocytosis and Leukopenia (43 pp.); IV. The Anemias (139 pp.); V. The Leukemias (49 pp.); VI. Hemorrhagic Diseases (24 pp.); VII. Miscellaneous (70 pp.); VIII. Hematologic Technic (59 pp.).

The section on Roentgenological Treatment of The Leukemic States was written by Dr. J. J. Clark; that on Blood Groups and Blood Transfusions by Dr. F. P. Parker; the Chapter on Malaria by Dr. E. Gambrell, and the section on Bone Marrow by Dr. R. P. Custer.

In Miss Garver, who prepared the drawings from which the colored plates were made, Dr. Kracke has found a collaborator of rare ability.

The book very properly begins with a discussion of hematologic nomenclature, which as everyone knows, is lamentably chaotic. This section deserves careful study for the recommendations made are sane and well based. A short glossary of hematologic terms will be welcomed by the technician and need not be ignored by the clinician.

The discussions of the origin and development of the blood bear ample evidence of extensive experience and a thorough and well balanced analysis of the literature. Indeed, this is characteristic of the book throughout. Whatever Dr. Kracke has to say is well and clearly said and readily understandable. In controversial matters his opinion is always fair, and obviously based on ample experience and a thorough consideration of all sides of the question.

The discussions of the various diseases of the blood are excellent—well phrased, understandable, free from pedantry, and bear the hall mark of the wide experience. The reader is conscious that while the author is not merely reiterating what others have said, he has utilized, adapted and interpreted it in the light of his own experience. If Dr. Kracke had not already had a reputation as a hematologist, these discussions would have given him one.

The section on Miscellaneous Conditions includes discussions of Infectious Mononucleosis, Polycythemia Vera, The Bone Marrow, Malaria, Blood Groups and Transfusions, and The Blood Picture of Normal Laboratory Animals.

So constant, and sometimes so great, are the changes in medicine in general and in its specialized phases in particular, and so constantly are the concepts of today modified and changed by the investigations of tomorrow, that seldom indeed may medical books be accepted as the last and final word.

This book, however, may be accepted as a well balanced, well written, and authoritative text on hematology well worthy of a place among the standard texts on this subject.

For the benefit of the inevitable future editions of this book one or two minor criticisms may be made. In view of the present frequency of transfusion as a therapeutic measure, the section on Blood Grouping and Transfusion seems somewhat brief. The discussion of blood grouping technic is somewhat abbreviated. There is no mention of the use of Type IV serum as a control procedure nor does it seem to this reviewer that the importance of titering grouping serum is sufficiently emphasized. This has been so clearly demonstrated by Coca's investigations that this might well be included for the benefit of those who prepare their own typing serum. Some mention of the various methods of detecting the intragroup anomalous agglutinins responsible at times

for delayed reactions might also be mentioned. And for the benefit of those called upon to explain their occurrence, the discussion of post transfusion reactions could be extended.

The section on technic embodies mainly the methods used in the authors' laboratories, although some others are mentioned. This reviewer believes that the Tallquist scale should be completely ignored as not only grossly inaccurate but archaic and personally prefers Haden's method to either the Dare or Sahli and hence regrets its absence.

These, however, are all minor criticisms.

This book may be heartily recommended without reserve to pathologist, hematologist, clinician, and laboratory worker alike.

EDITORIAL

The Laboratory and the Syphilis Problem*

A number of interesting developments have taken place in the laboratory diagnosis of syphilis since the beginning of the campaign against this disease by the Surgeon General of the United States Public Health Service. The reports of the Committee on the evaluation of serologic tests have been made public and some of the general conclusions are that the better flocculation tests are as reliable as the complement fixation tests, that *some* physicians can perform a given test as well or better than the originators of the test, that *some* private laboratories do excellent work others, not so good, and that there are State Board Laboratories which do inferior work in regard to these tests for syphilis.

Stimulated perhaps by these activities, serologists have brought forward a bevy of new tests, at least so-called new tests which for the most part are but slight modifications of previously reported tests, all claiming to be simplifications of some previous procedure. By inference or direct statement they imply that anyone from a janitor to a pathologist may perform the test—"an office test" is the general slogan.

Now none of this would be significant if it were not for the fact that particular emphasis has been placed on serologic tests in syphilis. The public and even the physician is being led to believe that a Wassermann reaction or its equivalent is all that is necessary to diagnose or rule out syphilis. The old slogan that the laboratory was but an adjunct to clinical medicine has long been forgotten, and it might be implied from a survey of recent literature that any technician may perform a ten-minute test and without other information condemn the patient to from two to five years of treatment, or release on the unsuspecting public

*Submitted for publication, September 13, 1937.

a potential focus of infection or a subsequent "case" for all too energetic social agencies.

What seems to be lacking in modern literature, especially that intended for the medical profession, is the fact that, while laboratory tests are of paramount importance in this disease, careful clinical histories, examinations and correlations are also important. Furthermore, behind these apparently simple tests are intricate and complicated foundations, the principles of which are but hazily understood. Why tests frequently go "off" is often never solved and it requires an experienced and fundamentally trained worker to appreciate when trouble exists, to say nothing of the need of such a person to straighten out the tangle.

Some advantage would seem to be had if investigators declared a holiday on the development of new tests for syphilis and spent their time in more fundamental research on the already existing more or less standard tests which are now as near perfection in regard to results as may be expected or even hoped.

The general state of affairs in regard to laboratory practice in syphilis is exemplified by a recent advertisement by a prominent biological reagent house. It advertised a certain "new" test which could be completed within ten minutes, which needed limited skill for its performance and which could be performed for an average cost per test of approximately two cents! The final thrust is certainly indicative of the present spirit. Where is the cost of equipment, overhead, assistants, time, education, and all the host of necessities for performing intelligently any laboratory test? Is the cost of the reagent the only cost?

Interesting also is the recommendation of the Committee on the evaluation of serologic methods in syphilis that State Laboratories be bolstered up to do better tests by increasing and training their personnel. If these laboratories are made bigger and better what will happen to the private practice of laboratory medicine? If these laboratories after all these years are still doing inferior work, one may reasonably ask why not reduce their activities instead of increasing them?

Perhaps it is time to take stock and see in which direction clinical pathology is being constructed. At least, it is time to examine the footings for cracks.

T. B. MAGATH.

THE SYPHILIS CAMPAIGN

In the campaign against syphilis the degree of recognition accorded the clinical pathologist will depend, in no small measure, upon himself. It is essential, therefore, that local and state groups shall prepare specific proposals covering those phases of the campaign which properly fall within the province of the clinical pathologist, and that such proposals shall then be discussed with county and state medical groups engaged in planning the campaign.

While such plans must necessarily vary somewhat in accordance with local conditions, it is quite probable that certain phases of one plan may be applicable to others. The Editor will be glad, therefore, to present for general information such plans as are submitted to him for the purpose.

In another place in this issue is given in full the proposals of the New Jersey State Society of Clinical Pathologists.

The section on "News and Notices" is open to summarized accounts of similar activities in other states.

R. A. K.

DIALOGUE ON THE RELATIONS OF GENETICS AND EXPERIMENTAL EMBRYOLOGY TO NEOPLASIA*

C. C. LITTLE AND STANLEY P. REIMANN

Dr. Little: Why do you suppose the Society of Clinical Pathologists has chosen a question of this type for discussion?

Dr. Reimann: Because pathologists realize that the basis of comparison for tumor growth problems is normal growth. Because they realize that many of their every-day problems in tumor diagnosis run into questions of inheritance, potencies, the way cells differentiate, the way they organize; in general in the way cells behave. They realize too that their specimens show cells only as of the instant when they are removed and fixed. They like to figure out how the cells arrived where they are—and what would have happened had the tissue not been removed when it was. In other words, in addition to the statics of pathology, they like to add the dynamic; in addition to cellular pathology, they want to think in terms of cellular physiology and when possible, even in terms of cellular chemistry. All of these and other questions, they realize, make it important that they know as much as possible of what has been learned and is being learned in such dynamic subjects as genetics and experimental embryology.

Dr. Little: This is interesting, Dr. Reimann, because in genetics we too are increasingly interested in the dynamic phase of that field. We find that experimental embryology is in one way the road over which genetics must travel in order to understand the relationship between a sub-microscopic structure known as the

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gene and the finished product of the adult character on which all of us clinical pathologists and biologists must base our classification.

Dr. Reimann: But Doctor Little, pathologists for many years have had the feeling that the genes alone will not solve some of their problems and it is with the greatest of interest that we learn from you that the tendency to tumor formation, at least in certain cases, can now be ascribed to transmission by way of the cytoplasm of cells. I believe pathologists would be interested in knowing how you determined that fact. You know that quite similar anatomic pictures, so far as chromosomes are concerned, are found in inflammatory conditions as in those that the pathologist calls real tumors. If healthy tumor cells are used for study, no change in the chromosome anatomy has been found in them specific of malignancy and no change which has not also been found in conditions other than malignancy, such as inflammations.

Dr. Little: I should be glad to talk about that briefly, Dr. Reimann, with the understanding that I am quite conscious of the fragmentary and preliminary nature of our knowledge of the topic. In the first place, the relationship between chromosomes and cytoplasm is one in which the chromosomes have certain definite advantages. Their characteristic structure, number, and distribution make a strong appeal to the natural desire of scientists to focus attention on what can be relatively easily seen and measured. The cytoplasm suffers because of its relatively obscure and variable type of organization and structure.

Geneticists naturally first described and have largely studied these characters which trace back to some real or hypothetical unit which can be correlated with the chromosome. They have found, however, in mammals that the vast majority of variations in form or structure cannot be wholly accounted for by that type of influence. Indefiniteness frequently appears in the picture. Many of us have therefore come to look upon the inherited or transmitted material other than the chromosomes as a

potential seat of new elements to be discovered. One such case appeared very strikingly in crosses made between strains of mice which differed greatly in the amount of cancer of the breast which they formed spontaneously. It was found that the influence of the mother upon the incidence of breast cancer in her female progeny was from seven to ten times as great as was that of the father. Since the female progeny inherited chromosomes equally from both mother and father it is evident that the greater influence of the mother depends upon something outside of the chromosomes. The simplest working hypothesis for the present is that this something may be inherent in or transmitted by the cytoplasm.

How about cases, Dr. Reimann, in which growth characters other than those influencing tumor formation show a cytoplasmic basis? Are there any such?

Dr. Reimann: There occurs to me immediately the question of sex and while this, strictly speaking, cannot be ascribed to cytoplasmic influence, nevertheless, it has been shown that sex destiny may be determined by factors other than chromosomes. In some animals there is a cyclic change of sex; thus the oyster is a she one year and a he the next year. It cannot be because the arrangement of chromosomes changes but because of some other factor. Then there is sex reversal. Thus, the famous rooster of Basle who started life as a perfectly good hen and after several years as an egg-layer and good mother began to grow a comb and spurs. Also he, she or it, greeted the morning like chanticleer. You will remember that this hen-rooster or rooster-hen was arrested, solemnly tried for witchcraft, and publicly burned at the stake. Surely in this beast and others which spontaneously change their sex, and in those in which it has been changed experimentally, the chromosome *x-y* complex has not been altered. The interest of pathologists in this lies in the fact that certain tumors exert definite influence on sex characteristics, such as the granulosa cell tumor and the arrhenoblastoma. Breast conditions are also influenced, such as in gynecomastia and certain benign tumors in both sexes.

It is plain, therefore, that chromosomes are not the sole determiners of those processes which determine this fate. The cell is a physiological unit and this in itself means that the cytoplasm also must enter actively into determination, because the functional unity of a cell depends on active reaction between nucleus and cytoplasm. It depends apparently on the viewpoint of the observer which part appears to predominate. Furthermore, we cannot ignore the localization problem. Inherited factors exert effects on development and differentiation, but this effect is often applied at a localized place. The individual is not a conglomeration of chemically different matter but is, in all senses of the word, an organism. Thus, if cytoplasm is indifferent in development, and if material particles of the chromosomes are the sole determiners of development, it is extremely difficult, if not impossible, to understand the typical localization of their action. But apart from such general considerations, experiments with bastardization have shown that twins result, for instance, in triton when half blastomeres are experimentally constricted and separated. If the plane of cleavage is in certain positions one blastomere develops into a new organism and the other into only a fragment. Notwithstanding the fact that the triton egg has great capacity to regulate itself, i.e., compensate, the nucleus of one of the half blastomeres cannot maintain itself even though, according to the genes idea, it contains all of the anlage material. This demonstrates activity on the part of the cytoplasm as well as a determining effect. Similar experiments have shown that the same thing holds true in certain insects. Perhaps, Dr. Little, you could give another example or two.

Dr. Little: Indeed I can, Dr. Reimann. I can mention the famous experiments of Wettstein on mosses in which leaf-shape inheritance and the growth habit inheritance is purely cytoplasmic. This is so well known that it is even in standard text books of botany. Then again chromosomes of one species of *Crepis*, one of the compositae, have been transferred to the cytoplasm of another without the second losing its cytoplasmic character. Similar experiments have been done on *Epilobium*,

another plant. Finally no one has as yet demonstrated chromatin, let alone chromosomes, in the so-called blue-green algae and yet they breed true to form. The pollen tube of *Pelargonium* carries no chloroplasts and the color of the leaf is therefore inherited maternally. I need not call your attention to the fact that bacteria have no nucleus in the ordinary sense of the word and no chromosomes. While they are said to mutate with considerable ease, nevertheless it is possible to breed them true.

Dr. Reimann: I am very glad to hear about this because, after all, a strict chromosomal theory of development is preformation. We no longer think in terms of the picture of the little man inside of the spermatozoon, he in turn having little men inside of his spermatozoa and so on ad infinitum as per the old idea of preformation, or mere unfolding out of preformed structures. I am afraid that complete dependence on the chromosomes is just another of these preformation ideas. If development is anything, it is certainly epigenetic; which means that as one part is produced, it influences and exercises effects on the oncoming parts as they are developed. Furthermore, the idea that both nuclear and cytoplasmic constituents play rôles in development gives more opportunity for fitting in the facts of potency to our theories. You know, Dr. Little, that pathologists for many years have worked very industriously attempting to trace back the origin of tumors. They have not been content to say that a tumor arises in such and such an anatomical situation, such as, let us say, the side of the tongue, but they must trace it back to a microscopic origin identifying such and such a cell or few cells as having been the point from which the tumor arose. As you know, an enormous amount of work has been devoted to this subject, but since neoplasia is a dynamic consideration and the material on which these studies have been made has been fixed and stained, it does not surprise you that there are differences of opinion and endless squabbles about the points of origin. I am so glad to hear you say that indefiniteness frequently appears in the picture, especially in mammals.

Dr. Little: The existence of indefiniteness does not by any means necessarily mean defeat. It really should be considered a stimulus to further study in many different fields. A good example of the value of such studies is the work done by Dr. D. F. Jones at New Haven. Dr. Jones is a plant geneticist. He works chiefly with Indian corn—or maize. In this plant there are many mendelian characters known to depend primarily upon genes. The relationship of these genes to one another has been carefully analyzed. An excellent place to study the variation in such genes and in other chromosomal characters is in the endosperm of the plant. As you know, the endosperm is derived from gametic tissue—that is to say, tissue in which changes in the chromosomes will at once be evident. Dr. Jones found that minor variations in the distribution of chromatin material were fairly frequent. These small changes in chromatin distribution often lead to defects or as geneticists call them, deficiencies. Usually they produced only pigment changes but in a few cases actual morphological variations appeared. These were either overgrowth, under development, or abnormal growth of tissues usually controlled or orderly. The bearing of these experiments on the theory of tumor origin in mammals is indirect but highly suggestive.

Dr. Jones' work is also of interest in its bearing on Cohnheim's theory of embryonic rests. What do you think, Dr. Reimann, is the present status of that theory—or rather, how do you see its application to the experimental work which you and your associates are doing?

Dr. Reimann: Suppose I put it this way: The fertilized ovum contains all the potencies necessary for the production of a whole new individual. As the descendants of this fertilized ovum display these potencies they turn into different types of cells and then organize together into different kinds of tissues, parts and organs. This indefiniteness which you mention can be correlated with several principles in experimental embryology, first, and very important, every cell which has not used up all of its potencies still has more than it normally expresses. For ex-

ample, ordinarily but one new organism arises from a fertilized ovum, but under certain circumstances two or even more may appear. Thus the quintuplets. And by separating the two cells of a divided fertilized sea urchin ovum it has been possible to obtain at least eight sea urchins from one single cell. This cell therefore has the potency of producing not one, but at least eight complete, competent sea urchins. Then there are the regulatory phenomena whereby in typical, so-called regulatory eggs, cells can be removed from very young embryos and the completed organism shows no defect. Thus, the potency of the cells remaining would normally have been exerted to produce a certain given part; nevertheless, when necessary, other parts can be produced. Even in the completed organism regenerative and similar phenomena show that more potency exists than is normally expressed, for regenerates themselves can regenerate, and regenerated regenerates can again regenerate in various organisms in various parts. This means, as far as the origin of tumors is concerned, that when they arise from cells which have not lost their potencies, different kinds of potency may be expressed.

Experimental embryology has shown also that when differentiation of a cell has proceeded to a certain point it can no longer retrace its steps, that is, it can no longer dedifferentiate. It is only in very primitive organisms and in primitive cells that dedifferentiation has been found.

A third point is that after a cell has reached a certain degree of differentiation it can no longer divide. Unfortunately degrees of differentiation have not as yet been measured quantitatively with any degree of accuracy. In the very nature of the problem you can see what a complicated task this measurement is, for each cell has its own type of differentiation and its degree. Is there anything contrary to the findings of genetics when, from the point of view of experimental embryology, we say (1) tumors arise from incompletely differentiated cells; (2) if incompletely differentiated they have more potencies than they express and (3) it is possible to have developed all sorts of differentiations from any particular, incompletely differentiated cell or a group

of them. Consequently instead of searching as was done in the past for the origin of this, that and the other tumor, may we not say that they arose from division-capable, therefore incompletely differentiated, cells, therefore with multiple potencies, and therefore with the possibility of producing different anatomic pictures? This, at least, focuses attention on what protoplasm can do and makes so much more interesting the newer experiments in transplantation, with organizers and so on.

Dr. Little: I see nothing contrary to genetics in the situation that you outline. Of course, we must remember that we have no real idea of the number or extent of the earliest stages of processes which, if continued, may give rise to neoplastic growth. It is entirely conceivable that there may be hundreds of centers of abnormal growth which become abortive before a critical or even a discernible disturbance in morphology takes place. Our methods of observation as commonly employed are very rudimentary and primitive. It would perhaps be reasonable to change the major premises which we make and query whether it is not remarkable that more centers of abnormal growth do not appear rather than that so many *do*. From a biologist's point of view the importance of "malignancy" as such is not so great. The real question is what can start growth and cell development independent of centralized control? What causes or allows cells to resume or to assume a type of growth which characterizes lower forms of life or the very early stages of mammalian ontogeny?

Dr. Reimann: The crux of the situation in malignancy from the pathologist's point of view is that while the cells may differentiate and thus produce good anatomic pictures of, let us say, glands, and physiologically, may differentiate even to the point of producing mucus or hormones as from particular testicular tumors, they nevertheless do not organize. From many considerations such as the fact that ofttimes in the very same environment, malignant cells and normal cells display differences when

growing side by side, the latter organizing but the former not, it appears that some sort of change has taken place within the cell which destroys its ability to organize and incidentally to differentiate properly. Since this change occurs in a somatic and not a sex cell it has been called somatic mutation. Of course we all realize that the word "mutation," as ordinarily used, means a change occurring within the cell which leads to the production of descendants differing from their forebears. Nevertheless in ordinary mutation, these changed cells do organize. There is this difference. In ordinary mutation organization takes place, in malignancy it does not. Could you tell us, Dr. Little, a few things about what has been learned in genetics about somatic mutation?

Dr. Little: The reference to Jones' work, already made, bears on this question and need not be repeated. It may be helpful to remember that the definition of a mutation is that it is a sudden and self perpetuating genetic change. It is obvious that a cell or cells which give rise to a neoplasm meet this definition. That is not the whole question, however. Such a genetic change may theoretically occur in (1) a gene, (2) a portion or block of a chromosome larger than a gene, (3) in a whole chromosome, (4) in a whole set of chromosomes, (5) in the cytoplasm. If it occurs in a cell other than a germ cell it is said to be "somatic" i.e. of the body. It is evident that the influences which cause such a change may be solely initiated by the introduction of external agents such as radiation or application of carcinogenic substances. Since the degree or extent of such external stimuli can be changed experimentally it is evident that they comprise one variable in the situation. There must also be considered the reaction of the cell to such stimuli. This reaction may be largely determined by genetics as expressed in the material composition and type of organization of the cell. It may also be modified by the relative proportion of various chemical secretions and stimuli produced in different types of internal environments within the animal's body. These in turn may depend

partly upon different genetic influences as expressed by various degrees of activity of enzyme activity or hormonal secretion. The situation is therefore complex but lends itself to and demands much more extensive investigation than it has yet been able to receive.

Dr. Reimann: This is indeed very interesting, Dr. Little, and so we may consider somatic mutation as a possible working hypothesis. Might I imagine a state of affairs such as this: I would like to picture a cell which can still do something, that is, which has not exhausted its potencies, as having in it chemical compounds with numerous open bonds. It has actually been shown that immature tissue has in it much more general and more labile groups of compounds than mature tissue. As a cell matures may we picture that those open bonds are satisfied in certain directions, but that the satisfaction of these bonds is not definitely fixed but merely guided along general lines by inherited characteristics? Thus, for instance, the cell must remain species specific, but within this wall it may do numerous things, and what it does depends on the environment, in the broad sense of the term, in which it finds itself; in other words, what building blocks are offered to it, and what physical forces are exerted upon it? Now when a cell becomes malignant it cannot utilize these building blocks nor does it obey the physical forces which ordinarily make it organize.

Dr. Little: Such a conception would be perfectly consistent with what geneticists have found. In fact the degree of elasticity, chemically speaking, possessed by its cells may well be the fundamental basis for such evolutionary differences as one finds between such fixed and inelastic types as insects and such versatile and adaptable types as mammals. It may not be an accident, therefore, that theoretical genetics have been chiefly advanced by studies in insects while the practical relationship between the fixed inherited elements and the more unpredictable phases of growth and development remain very largely a mystery in mammals and similar forms.

Dr. Reimann: It seems, therefore, Dr. Little, that we are face to face with the organization problem in cancer. Biology has gone through periods in which certain large problems have occupied the center of attention such as descriptive morphology, evolution and genetics. Do you not think that in the future the organization problem will come increasingly to the fore? Just why do organisms organize the way they do into replicas of their parents? After all, geneticists are hard at work on this, and experimental embryologists also. The more we pathologists see of malignancy the more subtle do the differences appear between normal and cancer cells and the more insistent does the organization problem become. A few words from you on this would surely be appreciated, and then perhaps you will be good enough to sum up a bit and indicate in a general way the direction in which this biological problem should be attacked and wherein we pathologists can help and wherein we can keep our eyes open for contributions from geneticists and others in this field.

Dr. Little: To sum up I should advocate the general adoption by all active in experimental pathology and other branches of experimental medicine of the principle of using known genetic stocks of animals. These will be homogeneous and predictable to the highest degree possible in mammalian material. Second I should advocate the variation under experimental conditions of the factors of internal environment such as sex, age, amount of internal secretion, nutrition, various degrees of exposure to stimuli, etc. Then, keeping these latter factors constant, I should begin to vary the genetic types of animals used. One could thus gain a much more accurate picture of the relative importance of genetic and other agents in the etiology of all growth phenomena including cancer.

THE ETIOLOGY OF ECLAMPSIA

A Preliminary Report*

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There have been no acceptable theories to explain eclampsia on a pathological and physiological basis. No complications of pregnancy that might be interpreted as eclampsia have been produced experimentally, and, so far as is known, this condition does not occur in animals. When the pathological changes which are found in human eclamptics are produced in pregnant animals, the pathological physiology underlying this disease may be determined.

It is our purpose in this preliminary report to give the results of experimental and clinical studies which we believe indicate that eclampsia is primarily due to a fetal hypometabolism, which is in turn secondary to a subclinical maternal hypothyroidism exaggerated by the increased metabolism occurring during pregnancy. One condition present in hypometabolic states, and which we believe is largely responsible for the pathological changes causing eclampsia, is a faulty metabolism of cholesterol. Evidence of this abnormal function is recognized by an increase in the amount of cholesterol present in the circulating blood.

Clinical studies have shown that there often occurs a hypothyroidism during pregnancy which is more severe in patients who subsequently develop eclampsia. Studies on infant blood at birth show that when maternal hypothyroidism exists fetal

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hypercholesteremia is also present, and the hypercholesteremia of the fetus is in turn responsible for the changes that occur in the placentae of eclamptic women.

HISTORICAL

Baumann and Holly, studying cholesterol metabolism in normal pregnant rabbits, found that there occurred a hypocholesteremia during the second and third trimesters and also that following thyroidectomy non-pregnant rabbits developed a hypercholesteremia. In pregnant rabbits, however, thyroidectomy at the end of the first trimester produced only a temporary hypercholesteremia followed by a hypocholesteremia in the third trimester. They further showed that the cholesterol content of the blood of feti or partial thyroidectomized and goitrous rabbits is elevated as much as 100 per cent above normal.

Meigs, Blatherwick and Cary injected cholesterol-binding dyes into the blood stream of pregnant rabbits and later were unable to demonstrate their presence in the fetal tissues and from their experiments concluded that cholesterol does not pass through the placenta.

Marine[5] and others[7] have reported that during pregnancy in rabbits there occurs an elevation of the basal metabolic rate of 30 per cent or more above normal. Marine also demonstrated in pregnant rabbits thyroidectomized at the end of the first trimester an immediate drop in the basal metabolic rate; however, in the third trimester the rate became elevated, just as occurs in normal rabbits, though not reaching so high a level.

Hughes determined the basal metabolic rates of pregnant women and found that those who developed symptoms of late toxemia had low metabolic rates most of which were much below normal while patients with rates of plus 10 or above throughout pregnancy did not develop symptoms of late toxemia.

Bartholomew and Kracke,[1] in a study of over one thousand placentae, found that the placentae with acute infarcts occurred in patients suffering from eclampsia and microscopically observed a severe endarteritis effecting the arteries of the placentae of eclamptic patients. The changes noted in these vessels were

of a degenerative nature and so similar to the changes seen in cholesterol-induced arteriosclerosis of the rabbit that they theorized that the changes in the placental arteries which resulted in infarction and eclampsia were due to the hypercholesteremia of pregnancy. They also produced pathological evidence of eclampsia in normal non-pregnant guinea pigs by the injection of placental split proteins derived from degenerating placental tissue.

MATERIALS AND METHODS

Briefly, the scope of our study of this problem was as follows: (1) To determine at frequent intervals the cholesterol content of the blood of a series of pregnant women and pregnant rabbits. (2) To also determine the cholesterol content of the blood of a number of human infants at birth and rabbit feti at term. (3) By experimental and clinical observations to attempt to correlate the relationship of maternal and fetal blood cholesterol to the activity of the thyroid gland and to the development of placental infarction and eclampsia.

The modified Blohr technique was employed for all blood cholesterol determinations. To determine the cholesterol content of placental tissue a cube of formalin-fixed tissue approximately 2 cm. square and weighing approximately 5 grams was removed from the most normal appearing part of the placenta. After grinding in a mortar with 20 grams of plaster of paris the powder was dried at 110° Centigrade, placed in an asbestos thimble and extracted for one hour with 40 cc. of chloroform. The cholesterol content of the extract was then determined colorimetrically just as is done in the determination of the cholesterol content of blood.

As suggested by Bartholomew and Kracke, the placentae before final examination were placed in 10 per cent formalin solution for at least four weeks. They were then cut in slices 1 centimeter thick and the cut surfaces examined for infarcts.

All rabbits were kept under the same conditions and were fed a commercial rabbit food, fresh lettuce and tap water.

EXPERIMENTAL OBSERVATIONS

The rabbits used in the following experiments may be divided into three groups depending upon the amount of thyroid tissue removed. No thyroid tissue was removed from the rabbits of Group 1, a particle thyroidectomy was performed on the rabbits of Group 2, and a total thyroidectomy was performed on the rabbits of Group 3. To avoid confusion the rabbits will be considered in the above groups.

Group 1

Blood cholesterol determinations were made on four normal rabbits before impregnation and at three day intervals throughout their pregnancy. Before pregnancy, and during the first trimester, the average cholesterol content of the blood was 90 mgm. per 100 cc. In the second trimester the cholesterol content of the blood dropped to 60 mgm. per 100 cc. The latter level was maintained throughout the third trimester. (Figure 1.)

The feti were removed by hysterectomy and blood was obtained from the fetal hearts for cholesterol determination. The average fetal blood cholesterol was 80 mgm. per 100 cc. The fetal thyroids were removed, weighed and examined. The average amount of thyroid tissue was 0.66 gram per litter. The fetal thyroid glands had a typical thyroid structure with patent acini lined by cuboidal cells and apparently filled with colloid.

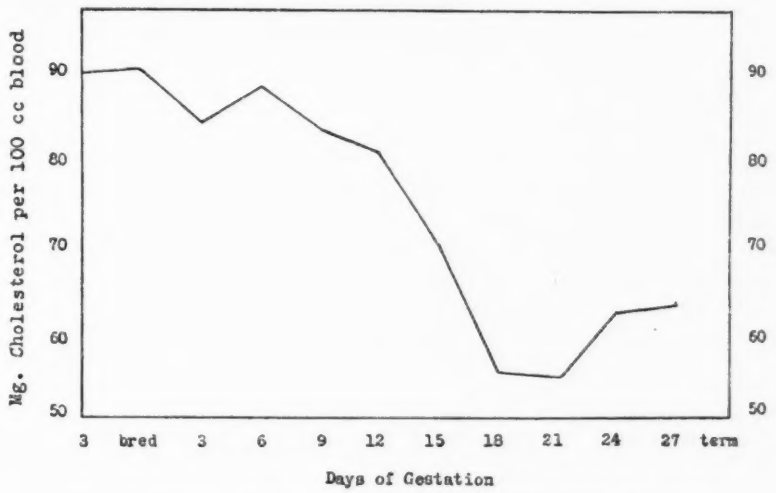

FIG. 1. BLOOD CHOLESTEROL DURING PREGNANCY, RABBITS

Group 2

Blood cholesterol determinations were made on seven normal pregnant rabbits at three day intervals. At the end of the first trimester of pregnancy both lobes of the thyroids were removed. We discovered later, when these animals were examined after death, that there remained in the neck of each animal aberrant thyroid tissue which almost equalled in bulk the amount of thyroid tissue previously removed. Obviously, it is difficult to perform a total thyroidectomy in the rabbit insomuch as the aberrant thyroid tissue increases in amount after thyroidectomy.

In this group of rabbits the blood cholesterol averaged 86 milligrams per 100

cc. of whole blood before pregnancy, but by the end of the first trimester had fallen to 73 mgm. per 100 cc. of whole blood. Immediately following thyroidectomy, which was performed at the end of the first trimester, the average blood cholesterol rose to 92 mgm. per 100 cc., but by the end of the second trimester the average cholesterol content had fallen to 69 mgm., and at term the average was 66 mgm. per 100 cc. It is apparent from these results that there occurred a temporary rise of the blood cholesterol following partial thyroidectomy. However, there later developed a hypocholesteremia, though not quite as marked as in normal pregnant rabbits at term.

At term the feti were removed by operation and the cholesterol content of fetal blood determined. In this group the cholesterol content of the fetal

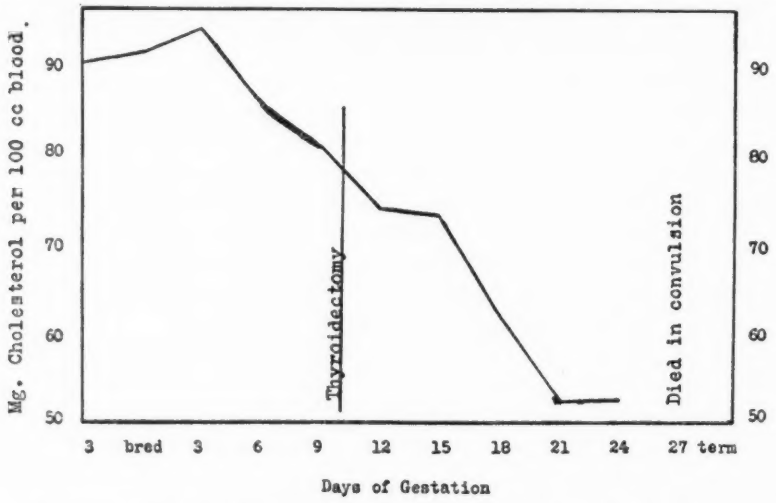

FIG. 2. BLOOD CHOLESTEROL DURING PREGNANCY, TOTAL THYROIDECTOMIZED RABBITS

blood was found to average 85 mgm. per 100 cc. of whole blood, which is approximately the same as that of feti of unthyroidectomized mothers.

The average amount of fetal thyroid tissue in each litter was 0.68 gram, which is approximately the same as that of a normal litter. Histological examination of these fetal thyroids revealed much normal thyroid tissue with colloid formation, although there was some evidence of increased activity as shown by a decrease in the amount of colloid and an increase in the size of the lining epithelial cells.

Group 3

This group of animals consisted of three normal pregnant rabbits treated and studied in exactly the same manner as the animals in Group 2 except that in this group total thyroidectomies were performed.

Before pregnancy the cholesterol content of the blood of the rabbits in this group averaged 92 mgm. per 100 cc. Total thyroidectomy performed at the end of the first trimester had no effect on the blood cholesterol, but by the first part of the third trimester the average blood cholesterol was 53 mgm. (Figure 2.)

Three days before term all the rabbits in this group developed convulsions and died. One rabbit delivered her feti and died six hours later. The other two did not deliver before death. Post mortem examination of the mothers revealed a total absence of thyroid tissue. No other gross lesions were found except thickened dark placentae. Microscopic examination of the maternal organs disclosed a toxic nephritis, moderate pulmonary congestion and an early toxic hepatitis.

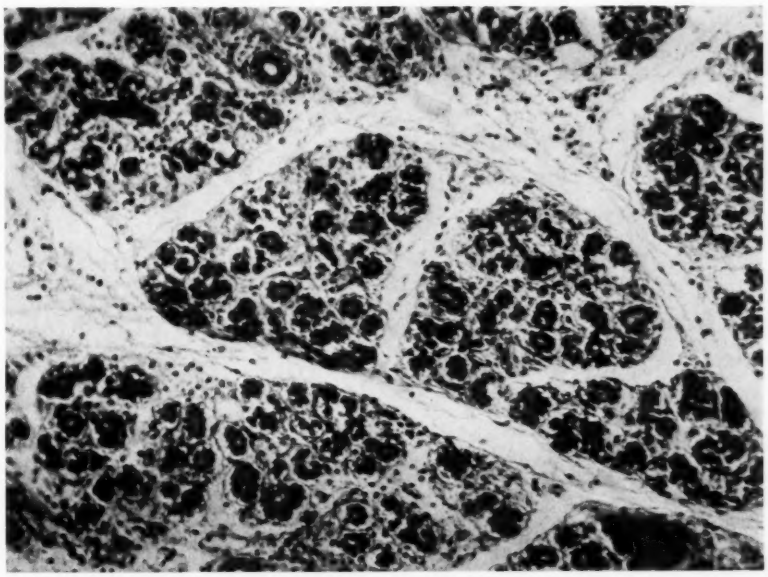

FIG. 3. FETAL THYROID FROM FETUS OF A THYROIDECTOMIZED MOTHER

Blood obtained from the feti immediately after the death of the mother was found to contain an average of 200 mgm. of cholesterol per 100 cc. of blood. This is two and one-half times the amount of cholesterol found in the blood of feti of normal mothers. The total amount of fetal thyroid tissue per litter averaged 0.30 gram although the number of feti was the same as in the normal and partially thyroidectomized animals. The fetal thyroids histologically showed extreme hyperplasia with no colloid formation. There was an increase in the interacinar cells and the epithelial cells normally forming the acini were grouped in clusters and their nuclei were large, swollen and pyknotic. Such an appearance, according to Marine[4], is definite evidence of hyperactivity. (Figure 3.)

FIG. 4. INFARCTED RABBIT PLACENTAE (CUT SECTION)

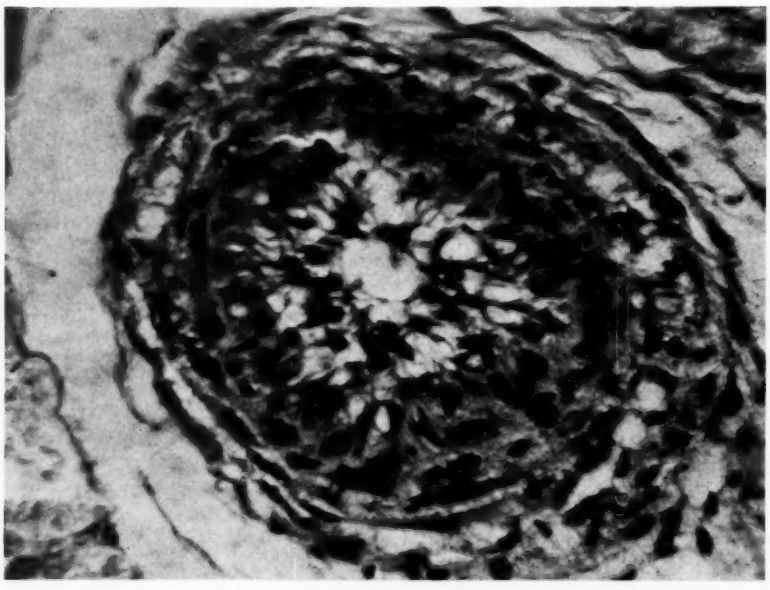

FIG. 5. ENDARTERITIS IN INFARCTED RABBIT PLACENTA

After fixation for four weeks the placentae of all the rabbits were studied. The normal rabbit placenta consists of two well defined parts, maternal and fetal. The maternal part is thin and in the fixed specimen contains no blood. The fetal part is thick and remains filled with blood after fixation.

The placentae of the rabbits of Group 3 were thicker than normal placentae. The maternal surface was considerably darker in color than the maternal surface of a normal placenta and also contained many dark red irregular areas. The line of demarcation between the maternal and fetal parts was not well defined and in the fixed specimen both portions were slate grey in color. The cut section of these placentae showed that beneath the dark spots on the maternal surface there were well circumscribed black areas which extended through the maternal portion and into the fetal part. There were also other circumscribed black areas in the fetal part that did not extend to the maternal surface. Grossly, these areas appeared to be infarcts. (Figure 4.)

Microscopic examination of the placentae of the rabbits of Group 3 revealed that the entire placenta was undergoing degeneration. The strands of tissue forming the stroma were swollen and stained very pale. There was very little or no blood in the fetal part.

The arteries of these placentae were found to have thickened walls and small lumina. Many arteries were found in which the intima was swollen to such a degree that there was complete occlusion of the lumen. This swelling of the intima was due to the presence of large fat cells in and beneath the intima and also to a diffuse round cell infiltration in this portion of the vessel wall. (Figure 5.)

CLINICAL OBSERVATIONS

As part of this study a clinical investigation was made in an attempt to determine the cause of the hypercholesteremia of human pregnancy and to determine the rôle blood cholesterol plays in placental infarction and the development of eclampsia.

Over one thousand cholesterol determinations were made on the blood of pregnant women at monthly intervals during the gestation period. The cholesterol content of the blood was found to average 175 mgm. per 100 cc. at the end of the second lunar month of pregnancy. A gradual rise occurred until at term the average was 247 mgm. per 100 cc. of blood. At the tenth day of the puerperium the cholesterol content of the blood averaged 243 mgm. per 100 cc. (Figure 6.)

Although the average cholesterol content of the blood showed an elevation at each lunar month of pregnancy, the cholesterol content of the blood of the individual patient showed wide variations. The same variation was present in the early puerperium. In many patients there occurred an elevation in the blood cholesterol until the seventh and eighth lunar months of pregnancy and then a drop in the ninth and tenth lunar months. Other patients showed a

steady elevation until the ninth month and a more marked elevation in the tenth month. Ten days postpartum the blood cholesterol frequently remained

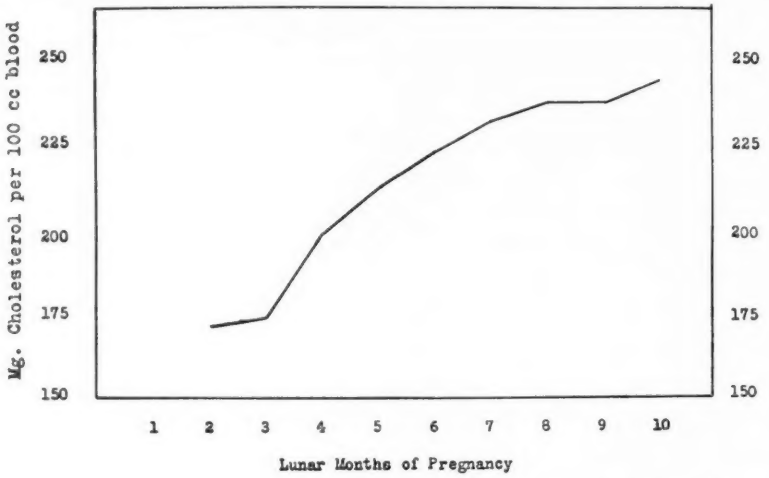

FIG. 6. BLOOD CHOLESTEROL DURING PREGNANCY (100 CASES)

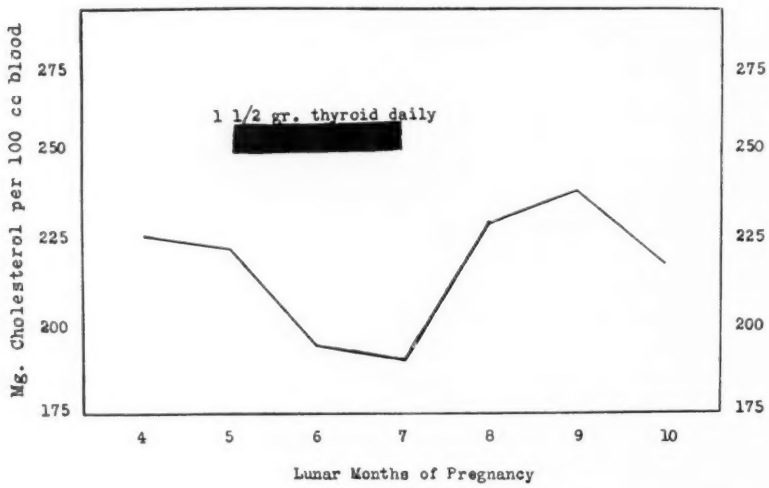

FIG. 7. THE EFFECT OF THYROID EXTRACT ON THE HYPERCHOLESTEREMIA OF PREGNANCY

the same as before delivery but in occasional instances there was a marked elevation while in others there occurred a drop.

The cholesterol content of the blood was always high in patients showing

symptoms of preeclampsia or eclampsia. If a patient developed eclampsia at the eighth lunar month of pregnancy the cholesterol content of the blood was just as great as in a patient developing eclampsia at term. Blood cholesterol determinations were made at four week intervals on twelve women who developed symptoms of preeclampsia or eclampsia. Twelve weeks before the diagnosis was made the blood cholesterol averaged 225 mgm. per 100 cc. of blood. There occurred a gradual rise until the termination of pregnancy when the cholesterol content of the blood averaged 268 mgm. per 100 cc.

Because hypothyroid states are accompanied by an increase in the cholesterol content of the blood thyroid extract was given to pregnant women in an effort to control the hypercholesteremia of pregnancy.

Blood cholesterol determinations were made on fifteen normal pregnant women during the fourth and fifth lunar months of pregnancy and then 1½ gr. of dessicated thyroid gland was given daily for sixty days. The cholesterol content of the blood averaged 224 mgm. per 100 cc. of blood at the end of the fifth lunar month when the thyroid therapy was started. During the sixth and seventh lunar months the blood cholesterol had dropped to an average of 197 mgm. per 100 cc. By the eighth lunar month and four weeks after the thyroid therapy had been discontinued the cholesterol content of the blood had increased to an average of 230 mgm. per 100 cc. By the ninth lunar month the blood cholesterol averaged 233 mgm. per 100 cc. By the tenth lunar month the average blood cholesterol was 218 mgm. per 100 cc. Ten days postpartum a further drop to an average of 212 mgm. per 100 cc. of blood had occurred. (Figure 7.)

The relationship of the cholesterol content of maternal blood during pregnancy to the cholesterol content of infant blood at birth was studied. If there had occurred a marked maternal hypercholesteremia during the third trimester of pregnancy, there also occurred an elevation of the infant blood cholesterol at birth. The cholesterol content of the infant's blood was always low if the cholesterol content of the mother's blood was not elevated. The patients studied were divided into two groups. The first group was composed of those infants in which the cord blood cholesterol was below 130 mgm. per 100 cc. and the second group those in which the cord blood cholesterol was above 130 mgm. per 100 cc. An average of 159 mgm. of cholesterol per 100 cc. of blood was found in twenty-three instances. An average of 110 mgm. of cholesterol per 100 cc. of blood occurred in twenty-seven instances. In the first group in which the cord blood cholesterol was low the maternal blood cholesterol at term averaged 223 mgm. per 100 cc. In the second group in which the cord blood cholesterol was high the maternal blood cholesterol at term average 265 mgm.

A study of 200 fixed placentae was made to determine the relationship of placental arterial disease and infarction to preeclampsia and eclampsia. The placentae of twelve patients suffering from eclampsia showed acute infarcts and extensive degeneration throughout the placenta. (Figures 8 and 9.) In the stroma of many of these placentae there were cystic cavities containing dark

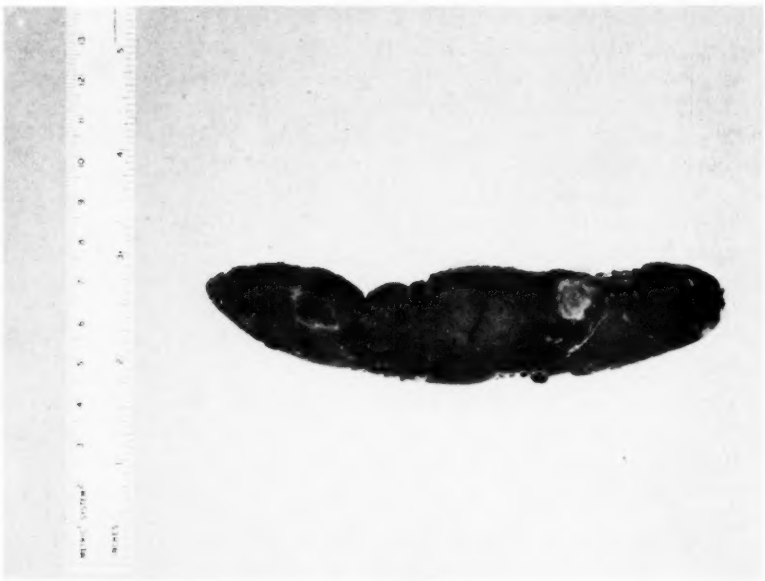

FIG. 8. PLACENTA OF A PREECLAMPTIC PATIENT

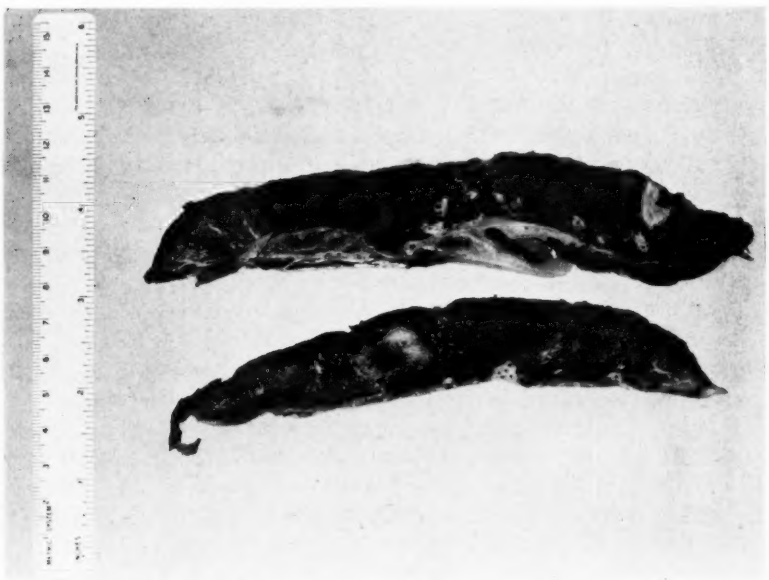

FIG. 9. PLACENTA OF AN ECLAMPTIC PATIENT

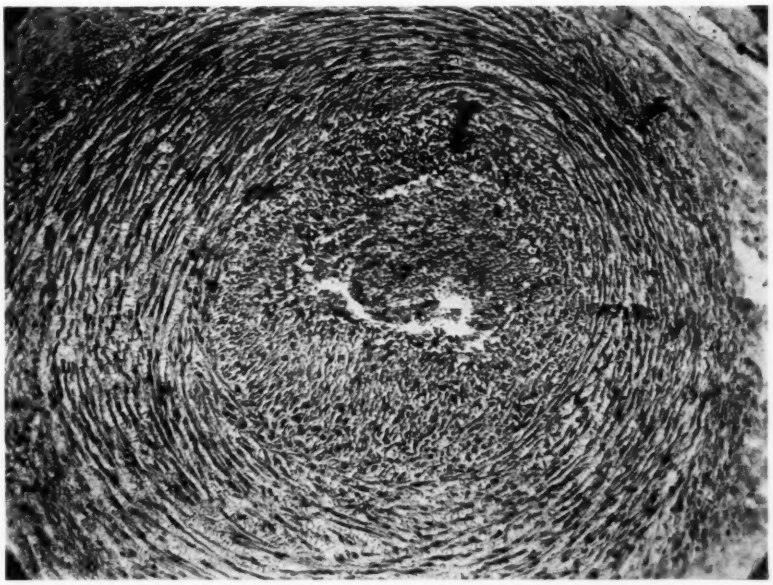

FIG. 10 ARTERIOSCLEROSIS IN PLACENTA OF ECLAMPTIC PATIENT

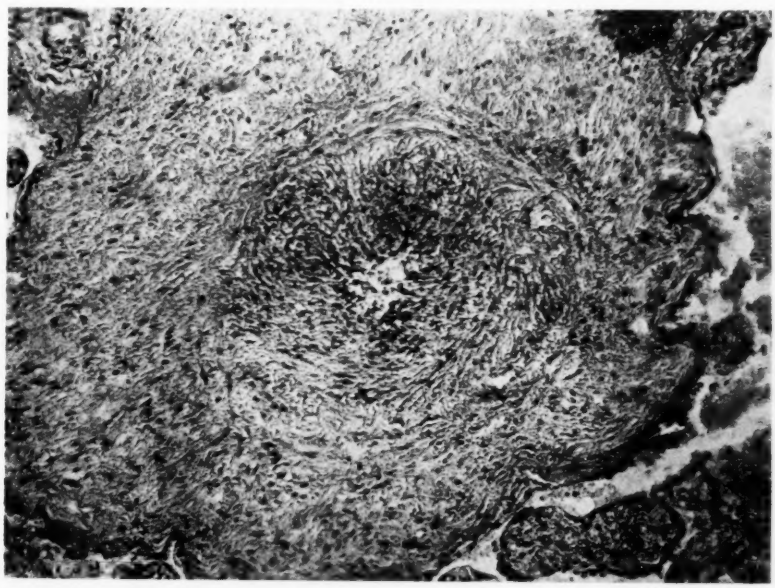

FIG. 11. ENDARTERITIS IN PLACENTA OF ECLAMPTIC PATIENT

brown fluid. In the infarcted placentae there was much fibrous tissue replacement of the stroma and thickening of the walls of the arteries. The placentae of patients with signs of late toxemia but who were not diagnosed as preeclampsia also showed occasional infarcts and degenerations but not to the extent of the eclamptic placentae. Small healed infarcts occasionally occurred in the placentae of patients without signs of late toxemia but acute infarcts never were found and there was very little degeneration of the placental stroma.

The microscopic examination of placentae from eclamptic patients revealed moderate to marked fibrosis of the placental villi. There was a marked perivascular fibrosis and endarteritis. (Figures 10 and 11.) There were abundant fat deposits in the arterial walls and beneath the intima. The endarteritis was of a degenerative type, the cells of the intima being swollen with fat deposits. There was also a round cell infiltration of the intima. In all placentae of preeclamptic and eclamptic patients arteries could be found in which the lumen was occluded by the swollen intima. In the placentae of normal patients that contained small healed infarcts there also occurred early arterial changes but not to the extent of eclamptic patient's placentae.

Cholesterol determinations were made on 135 placentae to determine if there occurred an increase in the cholesterol content of placentae from eclamptic patients. The tissue upon which these determinations were made was obtained from the most normal appearing part of the placenta and never an infarcted area. The average cholesterol content of 120 placentae of normal patients was 0.200 per cent and the cholesterol content of twelve placentae of patients suffering from eclampsia was 0.265 per cent. The placentae of eclamptic patients, therefore, contain approximately one-third more cholesterol than placentae of normal patients.

DISCUSSION

Blood cholesterol studies on pregnant rabbits revealed that, unlike human beings, rabbits develop a hypocholesteremia in the second and third trimesters of pregnancy. Total thyroidectomy in the non-pregnant rabbit produces a hypercholesteremia but in the pregnant rabbit has no effect on the blood cholesterol. The blood cholesterol of feti of total thyroidectomized rabbits was over 100 per cent higher than that of feti of normal rabbits. The thyroid glands of feti of thyroidectomized rabbits were in a state of extreme hyperplasia showing definite evidence of hyperactivity.

Because hyperthyroid states are accompanied by a low blood cholesterol and hypothyroid states by a high blood cholesterol, the variations of the blood cholesterol in normal and thyroidectomized pregnant and non-pregnant rabbits are interpreted as

showing the amount of thyroid secretion being used in the rabbit. During the second and third trimesters of pregnancy in a normal rabbit there is a maternal absorption of fetal thyroxin from many feti which affords an increase in maternal metabolism and a lowering of the blood cholesterol. If the maternal thyroid glands are removed after the fetal thyroid glands have developed sufficiently, the maternal circulation will absorb large amounts of thyroxin from the many feti and allow for an increase in maternal metabolism and a lowering of maternal blood cholesterol just as occurs in normal pregnant rabbits. This absorption of large amounts of thyroxin from the feti, however, produces a fetal hypothyroidism and hypercholesteremia. The fetal thyroid glands react to the fetal hypothyroidism by hyperactivity and hyperplasia, but are not able to maintain normal fetal metabolism. Because of the hyperactivity these fetal thyroid glands are not able to store colloid as are normal fetal thyroid glands. One can conjecture whether this hyperactivity during development may lead to permanent damage to the fetal thyroid.

Rabbits completely thyroidectomized during pregnancy died in convulsions shortly before term. Postmortem examinations revealed toxic hepatitis, toxic nephritis, pulmonary congestion and infarcted degenerating placentae. There was a severe endarteritis of the placental arteries producing occlusion. The endarteritis was of a degenerative type and was characterized by the presence of large fat cells in and beneath the intima and by a round cell infiltration of the intima.

Hypercholesteremia in the rabbit, produced by feeding a diet rich in cholesterol, leads to a cholesterol endarteritis indistinguishable from that found in infarcted rabbit placentae. Fetal blood flows in the arteries of the placenta, and placental infarction occurred only in the cases in which there was a fetal hypercholesteremia. There was a maternal hypocholesteremia in all instances in which placental infarction occurred. It is quite obvious, then, that the fetal hypercholesteremia produced the placental endarteritis which led to placental infarction. Animals in which this condition occurred subsequently developed convulsions and died.

Clinical studies show that eclampsia has a pathological and

physiological basis identical to the syndrome found in pregnant rabbits following total thyroidectomy. There is often a hypercholesteremia in the human during pregnancy and this is more marked in the women who develop eclampsia. The effect of the administration of thyroid extract indicates that the hypercholesteremia of pregnancy is due to a subclinical hypothyroidism that becomes exaggerated due to the increased metabolism for pregnancy. The single human fetus is unable to furnish sufficient thyroxin to combat maternal hypothyroidism, therefore, if maternal hypothyroidism exists before pregnancy it may become more marked during pregnancy as shown by the elevation of the cholesterol content of the maternal blood.

When there occurs maternal hypothyroidism and hypercholesteremia, the fetus will develop hypothyroidism and hypercholesteremia. If the amount of cholesterol in the fetal blood is elevated, cholesterol will be deposited in the walls of the placental arteries producing an endarteritis. If the endarteritis is severe the arterial lumen will be occluded resulting in placental infarction and degeneration. Toxins absorbed from the degenerating placental tissue produce a toxic state in the mother as shown by toxic changes in all of her tissues.

At the same time that fetal hypercholesteremia is producing placental arterial disease maternal hypercholesteremia may produce chronic renal disease. Therefore the toxins absorbed from a degenerating placenta will produce an acute toxic change superimposed on a chronic degeneration of the cells of the kidneys. If the resulting nephrosis is severe enough there will occur a renal shut down with anuria. This renal shut down combined with the state of general toxicity produces the syndrome known as "eclampsia."

After pregnancy the cholesterol content of the blood usually returns to normal as the thyroid is then able to maintain normal metabolism. If cholesterol deposits have produced a chronic nephritis during pregnancy the lowering of the blood cholesterol will allow the cholesterol deposits to be absorbed and kidney function will improve.

If maternal hypothyroidism is severe during pregnancy there

will be absorption of a large amount of fetal thyroxin. If this is the case, immediately after delivery the maternal thyroid may not be able to maintain metabolism at as high a level as was present before delivery. There would then occur a rise in blood cholesterol immediately after delivery. If there existed renal impairment before delivery the rise of blood cholesterol afterward will produce further kidney damage and if this is great acute renal failure may result producing eclampsia in the puerperium.

CONCLUSIONS

From the results of experimental and clinical observations we conclude that:

1. The hypercholesteremia of pregnancy is due to a subclinical hypothyroidism that becomes exaggerated due to the increased metabolism for pregnancy.
2. Eclampsia is primarily due to a fetal hypometabolism which is secondary to a maternal hypothyroidism.

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LATE METASTASIS IN PAPILLARY OVARIAN CARCINOMA*

Report of Case

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In February, 1934, an obese, white, married woman, 41 years of age, reported for treatment because of a subcutaneous tumor at the 4th left costochondral junction. The nodule was about 3 cm. in diameter, freely movable, not painful and it had been present about two months. As the patient was otherwise in good health, intervention was deferred.

In April, 1934, the nodule had increased a little in size and the overlying skin was slightly reddened and edematous. Its appearance suggested an abscess and aspiration was attempted but no fluid was obtained. The tumor was then surgically removed and the following pathological report made.

Gross description. The specimen is a broad-based, yellowish-white nodule 3 x 3 x 1.5 cm. The cut surface has a "bacon fat" consistency.

Microscopic examination. "Papillary cystadenocarcinoma of a type which might be metastatic from a papillary cystadenocarcinoma of the ovary. This is an unusual location for such a metastasis. Has the patient any history suggestive of ovarian tumor?"

Up to this time the patient's past history had not been obtained in detail, but it was now discovered that she had had two laparotomies. The old records revealed that in 1919, after 2 years of intermittent abdominal pain with the consciousness of a "bunch" in the abdomen, an operation had been done. The operative record described a "papilloma of the left ovary (? malignant). Removal of cyst and what could be removed of malignant portion." There was no microscopic study at this time.

In 1921, the patient reappeared complaining of pain in the right side of the abdomen, present since the previous operation, and of increasing size of the abdomen for the past six months. At a second operation, there was found "a tumor mass filling the pelvis, reaching 2 inches above the umbilicus on the right, adherent to the abdominal wall, omentum and intestines. Carcinomatous mass has broken through cyst wall. Pocket in cul-de-sac filled with cloudy fluid and carcinomatous masses." A complete hysterectomy was done. Again, there was no microscopic examination, but there seems little doubt from the

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description of the penetration of the cyst walls and the free solid tumor masses that the condition was a papillary carcinoma of the ovary.

Two months after the removal of the nodule in April, 1934, a second mass appeared just above the site of removal of the first. Under x-ray treatment this decreased in size until April, 1935, when it began to enlarge and pain in the left chest and arm began. X-ray examination of the chest at this time showed a mass beneath the sternum with foci of calcification.

On May 15, 1935, this mass was removed and was found to involve the region of the 3rd, 4th, and 5th ribs. There were also degenerated tissue masses

FIG. 1. ANTERIOR SURFACE OF SPECIMEN

in the mediastinum. The tumor was resected together with the anterior 3 inches of the 2nd, 3rd, 4th and 5th ribs and a corresponding section of the sternum. The patient responded well immediately after the operation, but the temperature failed to return to normal, and the patient expired on the 8th day following. Unfortunately, autopsy was not obtained.

DESCRIPTION OF TUMOR

As seen after removal, the tumor was about 10 x 10 x 5 cm. thick beneath the sternum. This portion was hard, white, and hyaline, with many foci of "sandy" calcification. The neoplasm penetrated between the 2nd and 3rd

FIG. 2. LATERAL VIEW OF SPECIMEN: ANTERIOR SURFACE TO LEFT

FIG. 3. POSTERIOR SURFACE OF SPECIMEN

ribs to form a soft, rounded, lobulated nodule about 5 x 5 x 3 cm. which was superficially well encapsulated, apparently growing in expansile fashion, and was the portion palpable through the skin (Figs. 1, 2, 3).

Microscopically, the superficial nodule showed quite typical papillary adenocarcinoma, practically identical with the material from the previous operation. The deep portion showed extensive hyaline degeneration and islands of carcinoma cells in various stages of degeneration, with foci of calcification and inflammatory reaction.

It is attractive to postulate that much of this hyaline change was due to x-ray treatment, and that the superficial growth was a development from an "uncaught" focus of malignant cells. Unfortunately, this hypothesis is hardly tenable for this particular tumor, because hyaline stromal change and the particular type of "psammoma" body calcification seen here is not uncommon in these tumors at any stage of their growth.

COMMENT

This case was deemed worthy of report because of the long duration of freedom of any evidence of tumor, 13 years from the second operation, 15 years from the first, and at least 17 years from the onset of symptoms. It is not clear whether the second tumor (1921) was a recurrence or whether the original tumor was bilateral. It seems that the latter may have been the case, as the original tumor was undoubtedly of the type of serous cystadenoma which is more often than not bilateral. No note was made of the condition of the right ovary at the first operation, but apparently the pelvis was pretty well filled with neoplastic tissue at that time. It is suggested that the contralateral ovary should be examined in every case of papillary cyst, for it is known that different degrees of malignancy may be found on the two sides, if two cysts are present.

The location of the metastasis is unusual, but not unreasonable, for the first apparently arose in one of the sternal lymph nodes draining the deeper structures of the abdominal wall above the umbilicus (2), and the second either via the deep lymphatics, or by extension through the diaphragm. The setting for either course was ready in 1921, when it was noted that the mass involved the peritoneum "two inches above the umbilicus."

In general, distant organ metastases are rare in tumors of the ovary. This was noted by Virchow[1] and is still in accord with

the general experience. A few late, distant metastases are reported by Stout[3]—"besides the opposite ovary, the peritoneum and the pelvic, omental, and supraclavicular lymph nodes, the only places we have noted metastases have been once each in the lung, the liver and the stomach." From other authors Stout quotes metastases in "the right arm, the spleen, a paratracheal node, bones, axillary nodes 5 years after removal of a cystic carcinoma of the ovary, without evidence of local reappearance, and a vaginal metastasis 7 years after removal of the primary ovarian tumor." In Stout's[3] experience, there were peritoneal or distant metastases in 90 per cent.

Prolonged life after the observed development of malignant characteristics in these tumors is the exception rather than the rule, but several single cases with very late metastases are noted by Stout: (1) supraclavicular lymph nodes 15 years after removal of ovaries—both involved; (2) cyst in spleen 25 years after; (3) omentum 16 years after (in this case 4 years of good health followed partial operation for these metastases and then the patient died suddenly with tumors in the liver, peritoneum, and bones)[3].

Recurrences at the site of operation in the peritoneum have been observed after 13, 15, 20 and 21 years, and in the skin incision after 21 years. The percentage of recurrences is given by Ewing[1] as 83.3 per cent as against 66 per cent for other types of carcinomas.

It should be noted that the apparent histologic innocence of a papillary cystadenoma is not infallible evidence that the disease is cured when the tumor is removed, for there is record of 16 per cent recurrences in such cases.[1] On the other hand, peritoneal implantations in these cases frequently regress after removal of the primary growth.

For the reason that the histology of these tumors is not always uniform within the same tumor, and that the small, thicker walled cysts may sometimes show malignant changes in their walls while the larger ones show only atrophic epithelium, the pathologist must examine all such material minutely, and for clinical purposes it is necessary to regard all cystadenomas of the

ovary, particularly those of serous or papillary type, as particularly prone to malignant change.

This case illustrates the importance of the microscopic examination of tissue, of a complete history, and of a well written operative description. The microscopic diagnosis was the key, and the operative record the open door to the whole story of events in this case, which may serve as a rather dramatic example of one of the many strange ways of the malignant process.

I am indebted to Dr. W. G. Hoebeke and Dr. Wm. Shackleton for permission to report this case, and to Dr. J. B. Jackson for the report of the x-ray examination.

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CHANGES IN THE BLOOD FOLLOWING REPEATED WITHDRAWAL OF ASCITIC FLUID IN CIRRHOSIS OF THE LIVER*

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The development of ascites in patients with uncomplicated portal cirrhosis is universally regarded as of serious prognostic significance and usually marks the progression of the condition into a terminal stage which Chapman, Snell and Rowntree[1] have termed the "decompensated stage," a designation which is not strictly accurate, since many of the most important functions of the liver may still be efficiently performed in many cases. The early literature of this subject contained frequent observations to the effect that withdrawal of the ascitic fluid appeared to hasten a fatal outcome in this disorder, and it was formerly the accepted practice to postpone paracentesis as long as possible. As stated by Rolleston and McNee[2], the chief reasons for this were (a) the occasional occurrence of peritoneal infection, (b) the fact that the removal of fluid entailed the loss of considerable amounts of protein and (c) the belief that the increased intra-abdominal pressure occasioned by the ascites might inhibit further transudation of fluid into the peritoneum.

This conservative attitude toward the removal of ascitic fluid in patients with cirrhosis is no longer generally maintained as it is usually felt that the ill-effects of its presence outweigh the possibly dangerous consequences of its withdrawal. This is particularly true in the case of individuals who are not too ill to be cooperative and who can benefit by improved dietary and

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other methods of management of hepatic disease. Occasionally, however, patients are observed in whom the institution of accepted dietetic and diuretic measures in conjunction with paracentesis appears to precipitate a rapidly progressive downhill course. The purpose of the present communication is to report certain chemical studies of the blood and ascitic fluid in 5 such cases.

MATERIAL AND METHODS

These patients varied in age from 38 to 56 years, marked ascites being present in each at or shortly after admission to the hospital. The diagnosis of portal cirrhosis was established in cases 1–4 at necropsy, case 5 being alive at the time of preparation of this report.

Blood was collected under anaerobic conditions before and at intervals after abdominal paracentesis, as indicated in table 1. Protein was determined by the method of Howe[3], chloride by the method of Whitehorn[4] and potassium by the method of Breh and Gaebler[5]. All patients when cooperative were maintained upon a fluid intake of 1600 cc. and a high carbohydrate diet containing approximately 0.49 gm. of sodium, 1.76 gm. of potassium and 0.74 gm. of chloride, as advocated by Rowntree, Keith and Barrier.[6] This routine diet could not be consistently maintained in any case in this series because of lack of cooperation due to abnormal mental state, vomiting, anorexia or diarrhea.

Every patient received, shortly after admission, ammonium chloride in doses of 5–10 gm. daily and a mercurial diuretic (salyrgan) intravenously or by rectal suppository every 3–4 days for several doses. This diuretic therapy was discontinued in each case because of the development of some indication of clinical regression during its employment.

RESULTS

Death occurred in cases 1–4 in from 20–42 days after initial paracentesis, which was performed once in case 3, twice in cases 2 and 4 and three times in case 1. Case 5 (6 tappings) is alive, but moribund, 147 days after the first paracentesis. In every instance, several of the following symptoms appeared during periods of diuresis induced by ammonium chloride and mercurial diuretics: vomiting, restlessness, insomnia, muscular twitching, tremors, clonic convulsions, delirium, weakness, mental depression, somnolence, stupor, coma. One or more of these persisted following cessation of diuretic therapy, death occurring in coma in each instance.

No unusual urinary or other indications of significant renal change were observed, the non-protein nitrogen of the blood being within normal limits (23–36 mgm. per 100 cc.) in each case. The urea clearance ranged from 63 to 84 per cent of the average normal. Clinical jaundice was present only in

TABLE 1

CASE	DATE	HEMATO-CRIT	B.P.	SERUM			ASCITIC FLUID						
				Protein	NaCl	K	Date	Amount	Protein		NaCl	Total protein removed	Days
									Per cent	Total			
				grams per cent	*mgm.*	*mgm.*		*cc.*		*grams*	*mgm. per cent*	*grams*	
1. D. G.	10/27/36	34	132/90	6.3	622	20.4							
	11/ 3/36			6.2	595		11/_3/36	7,000	2.5	175	623		
	11/ 5/36	37		5.4	611								
	11/12/36			7.1	564	26.3	11/12/36	6,000	1.8	108	712		
	11/15/36			5.9	532							526	26
	11/29/36			5.8	542		11/29/36	9,000	2.7	243	704	(20)	
	12/ 2/36	42	104/64	5.4	502	32.1							
2. R. A	11/18/36	38	126/92	6.8	604	16.1	11/18/36	9,000	0.9	81	628		
	11/21/36			6.2	540							301	34
	12/22/36			5.7	580		12/22/36	11,000	2.0	220	640	(9)	
	12/25/36			5.1	521								
	12/30/36	44	98/66	5.8	498	29.4							
3. E. O.	12/ 9/35	40	100/80	5.3	610	21						266	16
	12/23/35			4.8	560		12/23/36	7,000	3.8	266	634		
	12/26/35	41	92/70	4.2	514	29.3						(17)	
4. T. N.	8/ 3/35	36	124/82	5.6	563	18.1							
	8/12/35			5.4	572		8/12/36	6,000	2.4	144	620	195	21
	8/15/35			5.1	543							(9)	
	9/ 2/35			3.5	506		9/ 2/36	8,500	0.6	51	602		
	9/ 5/35	36	106/74	3.6	500	32.4							

	9/ 5/35	36	106/74	3.6	500	32.4							
5. C. Y.	12/15/36	32	140/90	7.4	614	17.1							
	1/ 4/37 1/ 7/37	27		6.8 6.1	564 574		1/ 4/37	6,000	1.3	78	564		
	1/13/37			7.8	595	21.0							
	2/ 2/37 2/ 5/37			6.2 5.8	592 614		2/ 2/37	12,000	2.1	252	686	736 (6.5)	113
	2/15/37	23	110/70	6.8	580	20.4							
	3/ 1/37			6.0	578								
	3/11/37 3/14/37			6.1 5.4	594 612	26.2	3/11/37	8,000	2.2	176	660		
	3/24/37 3/27/37			5.2 4.5	581 567		3/24/37	6,000	1.8	102	673		
	4/ 5/37 4/ 8/37			6.2 6.1	602 625		4/ 5/37	4,000	0.5	20	667		
	4/29/37			5.8	608	29.3							
	5/21/37 5/24/37	18	90/60	6.1 6.5	553 574	32.3	5/21/37	9,000	1.2	108	617		

case 2 (serum bilirubin 3.4 mgm. per 100 cc.) but some degree of elevation of serum bilirubin (1.2–1.9 mgm.), abnormal bromsulphalein retention (10–40 per cent) and excessive urobilinuria (1:40–1:100) was observed at some time in every case.

Every patient in this series presented the picture of advanced malnutrition. During the last few weeks of life little food was taken voluntarily, and administration of fluids by nasal tube and parenterally was necessary. Demonstrable subcutaneous edema was noted only in case 4, during the last week of life (serum protein 3.5 gm.). The plasma CO_2 combining power in cases 1–4 ranged from 47 to 72 volumes per cent shortly before death, which occurred within 3 days after the date of the last findings recorded in table 1.

Although considerable local relief followed tapping in most instances, toxic manifestations, particularly those referable to the central nervous system, appeared to be aggravated. Persistent vomiting was a troublesome symptom in cases 1–4. In every instance the abdominal fluid reaccumulated rapidly and the blood pressure fell progressively. The pertinent details as to hematocrit values and chemical findings are presented in table 1.

DISCUSSION

Fortunately, data presented in this report are by no means representative of those usually obtained in patients with portal cirrhosis, many of whom respond favorably to similar methods of management. However, far too little consideration is usually given to the general metabolic status of the individual patient in directing therapeutic efforts toward the amelioration of a dominant presenting clinical manifestation, such as ascites. Analysis of observations in this series of patients reveals certain features bearing upon this point.

Protein

The concentration of protein in the ascitic fluid varied from 0.5 to 3.8 gm. per 100 cc., being above 2 gm. per cent in 6 of the 14 specimens examined. These values, in the absence of evidence of active peritoneal inflammation, are somewhat higher than those reported by Loeb, Atchley and Palmer[7], Foord, Youngberg and Wetmore[8] and Myers and Keefer[9]. They may be due to a higher relative rate of reabsorption of water as compared to protein from the peritoneal space, a factor which must vary considerably from time to time. The total amount of pro-

tein removed by paracentesis varied from 195 gm. over a period of 21 days (case 4) to 736 gm. in 113 days (case 5), the average daily loss by this route being approximately 6.5 (case 5) to 20 gm. (case 1). The quantity removed at a single tapping ranged from 20 to 266 gm.

The serum protein concentration was within normal limits in 3 cases at the time of admission, despite the advanced nature of the disease and the state of marked malnutrition exhibited by all. Subnormal values were obtained in all cases at some time during the period of observation. This is in accord with many reports by other observers, among the most recent of which are Snell[10] and Myers and Keefer[9]. The serum protein concentration 48–72 hours after paracentesis was 0.6–1.2 gm. per cent lower than at the time of tapping in 7 instances and 0.1–0.4 gm. per cent lower in 3 instances. In 2 instances the post-paracentesis values were 0.1 and 0.4 gm. per cent higher than previously. Occasionally, a rather marked secondary increase in the serum protein concentration was observed, occurring within a remarkably short period of time (case 1: 5.4 to 7.1 gm. in 7 days; case 5: 6.1 to 7.8 gm. in 6 days; 5.8 to 6.8 gm. in 10 days; 4.5 to 6.2 gm. in 9 days). In every case however, particularly in cases 1–4, despite these minor fluctuations, the serum protein concentration tended to diminish steadily throughout the period of observation.

The basis of these changes in serum protein is conjecturable. Myers and Keefer[9] found a decreased concentration 12 hours after paracentesis, coincident with spontaneous marked diuresis and decreased peripheral edema. They attributed the decrease in protein concentration to possible dilution of the blood plasma. This explanation does not seem applicable here because of the rapid reaccumulation of ascitic fluid and the absence of diuresis during this period. It seems more likely that the primary fall in serum protein concentration is dependent largely upon an actual loss of protein from the plasma into the peritoneal collection of fluid. The importance of this factor is minimized by Snell[10] on the basis of data presented by Barnett, Jones and Cohn[11]. It would appear that too little consideration is given

to the influence of possible alterations in hemoconcentration, as indicated by the studies of Bernard[12], in interpreting the occasional preservation of comparatively normal serum protein levels in such cases.

It seems significant that the rather marked and relatively rapid secondary increase in serum protein concentration observed on several occasions in the present series occurred in each case during a period of active diuresis and increasing ascites, the former being induced by ammonium chloride and salyrgan. In view of the findings of several investigators, including Knutti and his associates[13] and Myers and Keefer[9] regarding the defective formation of blood proteins in the presence of advanced hepatic disease, and the necessarily inadequate protein intake of these patients, it scarcely seems likely that this increased serum protein concentration can be attributed to active new formation or to replenishment from pre-existing stores in the body. It is possible that it may be due in part to reabsorption of a portion of the ascitic fluid protein into the blood stream by way of the lymphatic circulation. However, under the existing circumstances of inadequate fluid intake and the rapid loss of fluid from the blood and tissues into the peritoneum and by diuresis, it would appear most probable that the secondary increase in serum protein concentration is dependent, in large part at least, upon hemoconcentration. This explanation is supported by the absence, in cases 1–4, of a progressive fall in erythrocyte count and hematocrit value despite the presence of ideal conditions for the development of progressive anemia. In fact, the hematocrit values increased steadily in cases 1 and 2 and remained essentially unchanged over periods of 17–33 days in cases 3 and 4. The progressive anemia in case 5 may be attributable to the much longer course and consequently longer period of malnutrition. Acceptance of the hypothesis of hemoconcentration would, of course, imply the existence of a much more marked degree of plasma protein depletion than is indicated by its observed concentration. Such would, in fact, be much more consistent with the loss of the relatively large quantities of protein removed from these patients by abdominal paracentesis

in the face of probable defective reformation of plasma protein incident to advanced hepatic disease.

Chloride and Potassium

The ascitic fluid chloride concentration (expressed as sodium chloride) ranged from 564 to 712 mgm. per 100 cc. The total amount removed in individual cases varied from 45 gm. in 16 days (case 3) to 222 gm. in 113 days (case 5), an average loss of about 2–6 gm. daily by this route. The serum chloride concentration prior to paracentesis ranged from 563 to 622 mgm. per 100 cc. and no consistent change was observed 48–72 hours after paracentesis. However, cases 1–4 exhibited a steady fall in serum chloride concentration to terminal levels (within 72 hours of exitus) of 498–514 mgm. per cent. Obviously, because of the existing state of hemoconcentration, the observed diminution in serum chloride concentration does not adequately indicate the extent of chloride depletion. This is undoubtedly contributed to by dietary chloride restriction, diuresis, vomiting, diarrhea and the accumulation of fluid in the peritoneal space with its rapid reaccumulation following paracentesis. Sodium determinations were not made, but it is probable that changes in this element were of the same order as those in chloride.

The serum potassium concentration prior to the institution of treatment ranged from 16.1 to 20.4 mgm. per 100 cc. A progressive increase occurred in every case, to terminal values of 29.4–32.4 in cases 1–4. The serum potassium in case 5 was 32.3 mgm. per cent 147 days after initial paracentesis. The cause for this increase is not readily apparent. It may have been contributed to by the relatively high potassium intake (as compared with sodium) and by the state of advanced malnutrition, with probably relatively rapid tissue wasting.

The facts may be purely coincidental, but are nevertheless suggestive, that in cases 1–4 the simultaneous development of hypochloremia (and probably low serum sodium) and hyperpotassemia was accompanied by clinical manifestations of rapidly progressive toxemia, which terminated fatally in a very short time. In case 5, with hyperpotassemia but no hypochloremia,

these toxic manifestations, although present, were not nearly as severe nor as rapidly progressive. The recent report of Scudder, Zwemer and Truszkowski[14] of an increase in serum potassium in experimental and clinical acute intestinal obstruction supports the view suggested by the present observations that hyperpotassemia may be a factor of clinical significance in conditions other than primary adrenal insufficiency. In any event, it would appear that the data presented indicate the existence, in the patients studied, of marked plasma protein depletion and of a profound disturbance of water balance and electrolyte distribution, characterized by hemoconcentration, hypochloremia (and probably diminished plasma sodium concentration) and hyperpotassemia. These changes, under the conditions existing in certain cases of portal cirrhosis, appear to be exaggerated by certain accepted routine therapeutic procedures, such as repeated paracentesis, restriction of fluids, chloride and sodium, a relatively high potassium intake and active diuretic measures, such as the administration of ammonium salts and salyrgan.

SUMMARY AND CONCLUSIONS

Studies of the blood and ascitic fluid in 5 patients in the terminal stages of portal cirrhosis with rapidly reaccumulating ascites revealed the following:

1. A loss of protein, by removal of ascitic fluid, of from 195 gm. in 21 days to 736 gm. in 113 days, the average daily loss being 6.5–20 gm.

2. The serum protein concentration tended to diminish immediately after paracentesis and to increase subsequently. The primary fall was attributed, in part at least, to the loss of protein from the blood into the rapidly reaccumulating peritoneal effusion; the secondary increase was attributed to progressive hemoconcentration, which was also suggested by hematocrit determinations. In the 4 fatal cases the serum protein concentration diminished during the period of observation, the actual degree of protein deficit being masked by hemoconcentration.

3. The quantity of chloride (expressed as sodium chloride) lost by paracentesis averaged 2–6 gm. daily. In the 4 fatal cases

the serum chloride concentration fell to distinctly subnormal levels, the actual degree of chloride deficit being masked by hemoconcentration.

4. In each case the serum potassium concentration increased from within normal limits originally to 29.4–32.4 mgm. per 100 cc. at the end of the period of observation.

5. It is suggested that the marked disturbance in water balance and electrolyte distribution may have some bearing upon the development of toxic manifestations in patients with advanced portal cirrhosis and ascites.

The clinical condition of these patients appeared to be made worse by the institution of a form of therapy effective in less severe cases. This included repeated paracentesis, diuresis induced by ammonium salts, and a high carbohydrate, low sodium, low chloride and relatively high potassium diet, with moderate fluid restriction. This therapeutic procedure would appear to contribute to and to exaggerate the serum protein deficit, hemoconcentration, hypochloremia and hyperpotassemia observed in the patients in this series.

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FROZEN-SECTION BIOPSY AT OPERATION*

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The purpose of this inquiry was to study the diagnoses made on forty-five cases of suspected malignancy by frozen-section while the surgeon waited on the operating-room, and to compare them with the diagnoses made on the same cases after deliberate study of carefully prepared paraffin sections stained with hematoxylin and eosin and permanently mounted. It was made with a view to evaluating the dependability of a diagnosis made under the circumstances mentioned and the advisability from the standpoint of maximum safety for the patient of recommending the procedure as a diagnostic measure and a basis for surgical judgment.

The work was carried on at the Bryan Memorial Hospital. The cases were consecutive, and selected for the one feature above mentioned, namely, that the surgeon removed a fragment of the suspected tissue for biopsy at a surgical operation, interrupted the operation pending diagnosis, and waited in the operating-room with the patient under an anesthetic until he received the report from the laboratory to use as a basis for his decision as to choice of method for finishing the operation.

The minimum time during which the operation was delayed was 10 minutes; the maximum, 30 minutes; the average 14 minutes. Variations were due to: (1) unexpected minor technical difficulties; (2) varying lengths of time required for the stain to differentiate and clear properly in different tissues; (3) varying amounts of time required for the pathologist to make up his mind what to report to the surgeon.

The tissues were frozen fresh, and stained with polychrome-

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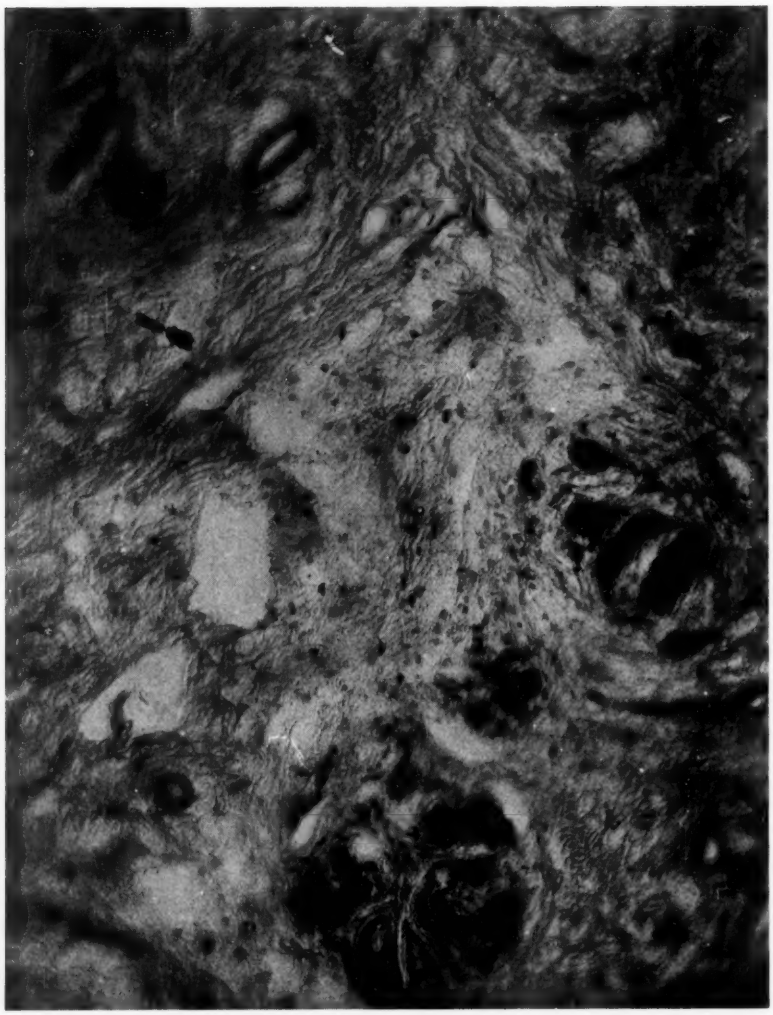

FIG. 1. CHRONIC MASTITIS

Vital stain with polychrome toluidin-blue on frozen section. 10 × ocular and 4 mm. objective. Commercial ortho film and K 3 filter with Mazda flood-bulb.

toluidin-blue without fixing. The technic of staining was as follows:

1. Float out the section on a slide, handling it in a stream of water and a pipette rather than with glass or metal hooks.
2. Remove the water from around the section by pipette or blotter.
3. Flood with polychrome-toluidin-blue for one minute.
4. Blot off the stain and cover with distilled water.
5. Remove the water and replace with a fresh supply, just enough to support a coverglass.
6. Apply coverglass.
7. Allow to stand until the section clears in the water sufficiently to permit examination and until colors differentiate (5 to 10 minutes).

When the staining and clearing are complete there is a contrast between the basophilic and acidophilic structures, the former staining blue and the latter red. The slide is available for study until the water dries (about 30 minutes). No method has as yet been found to preserve permanently sections thus prepared.

The differentiation in the tissue is of good degree, and comparable to that in a hematoxylin-and-eosin stained section. Necessarily the sections are not as thin as could be obtained by paraffin embedding and section on a heavy microtome. The differentiation of protoplasmic structures is not up to research requirements. One especially desirable diagnostic point, the distribution of chromatin in the nucleus, is poorly brought out by this method. It requires some practice to become familiar with the vital-stain appearances, but after this is once acquired, the appearances are typical and not difficult to recognize and the preparations are satisfactory for clinical diagnostic purposes. Whatever diagnostic errors may occur in the frozen-section-at-operation method cannot be blamed entirely upon the appearance of the vital-stained section in comparison with that of the permanent preparation stained with hematoxylin-and-eosin.

In order to confirm this statement, the following observations were made. A block of the suspected tissue was cut in two and one-half of it preserved in the refrigerator in a frozen state. The other half was embedded in paraffin, and permanent, hematoxylin-eosin-stained slides were made from it. The first half was then cut with the freezing microtome, stained with the vital stain and the sections prepared by the two methods were

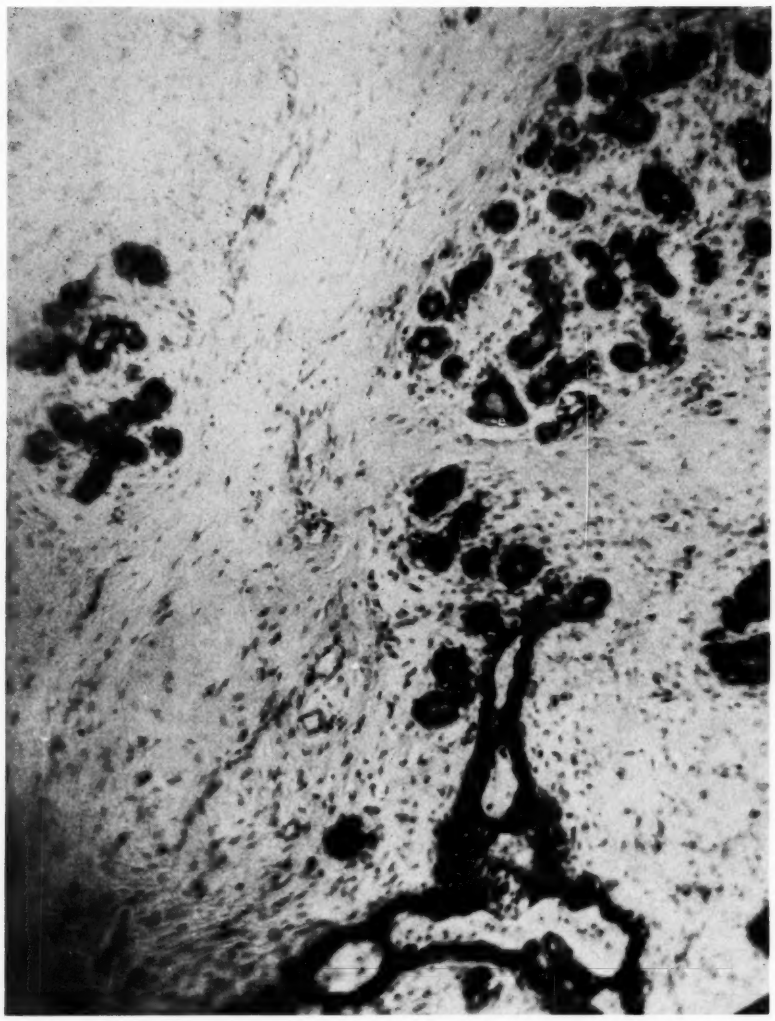

FIG. 2. CHRONIC MASTITIS

Hematoxylin-eosin stain on paraffin section. 10 × ocular and 4 mm. objective. Commercial ortho film and K 3 filter with Mazda flood-bulb.

then compared side by side. No essential differences which might interfere with diagnostic reasoning were found.

This work was done upon the cases of nine surgeons. The distribution of the cases numerically being as follows:

SURGEON	NUMBER OF CASES	SURGEON	NUMBER OF CASES
A	30	F	2
B	3	G	1
C	3	H	1
D	2	I	1
E	2		

As there are twelve other surgeons operating at this hospital whose work is quantitatively of the same order of magnitude as that of the one who had the 30 cases, the figures would suggest that this one man was especially interested in the method. There was no prearranged plan of study between the writer and any of the surgeons.

The surgeons all expressed spontaneous willingness for the pathologist to take plenty of time during the operation to do his work well. Whatever errors occurred in diagnosis cannot be blamed upon pressure from the surgeons at the time of operation.

The clinical factors taken into consideration in making the diagnoses, and occurring with sufficient uniformity to permit of tabulation, were the age of the patient and the gross description of the lesion. Other clinical data were either not available in the records, or were so scattered as to prevent statistical study.

The age groups of the patients follows:

AGE GROUP	NUMBER IN GROUP
years	
20 to 30	3
30 to 40	7
40 to 50	14
50 to 60	14
60 to 70	6
70 or over	1

The average age of the patients was 48.2 years; the mean age was 45 years. These figures represent the ages at which clinicians took most seriously the problem of deciding between malignancy on the one hand, and the undesirability of sacrificing tissue unnecessarily on the other. The figures above and below

these ages fall into a curve, which corresponds as nearly as the small number of cases will permit, to the curve of normal probability. That is, mathematically it states that at earlier ages, clinicians do not find malignancy as frequent, and at later ages they operate less upon it.

The organs examined are listed below, with their general diagnoses:

	TOTAL	MALIGNANT	NON-MALIGNANT	BORDERLINE
Female breast	31	20	10	1
Uterus	7	1	5	1
Ovary	1	0	0	1
Gall-bladder	1	0	1	0
Male breast	1	0	0	1
Appendix	1	0	1	0
Neck	1	1	0	0
Leg	1	0	0	1
Total	45	22	18	5

The term "borderline" as used here signifies that the pathologist was unable to make up his mind categorically whether to class the lesion as malignant or benign but considered that there was sufficient possibility of its being malignant to justify placing it under suspicion.

The following table contains a descriptive list of the specimens as received in the laboratory, with their general diagnoses:

LESION	MALIGNANT	NON-MALIGNANT	BORDERLINE	TOTAL
Placque	5	1	3	9
Fragment	2	0	0	2
Nodule	5	5	0	10
Infiltration	3	3	0	6
Mass	7	2	1	10
Curettings	0	4	1	5
Mass of veins	0	1	0	1
Total	22	18	5	45

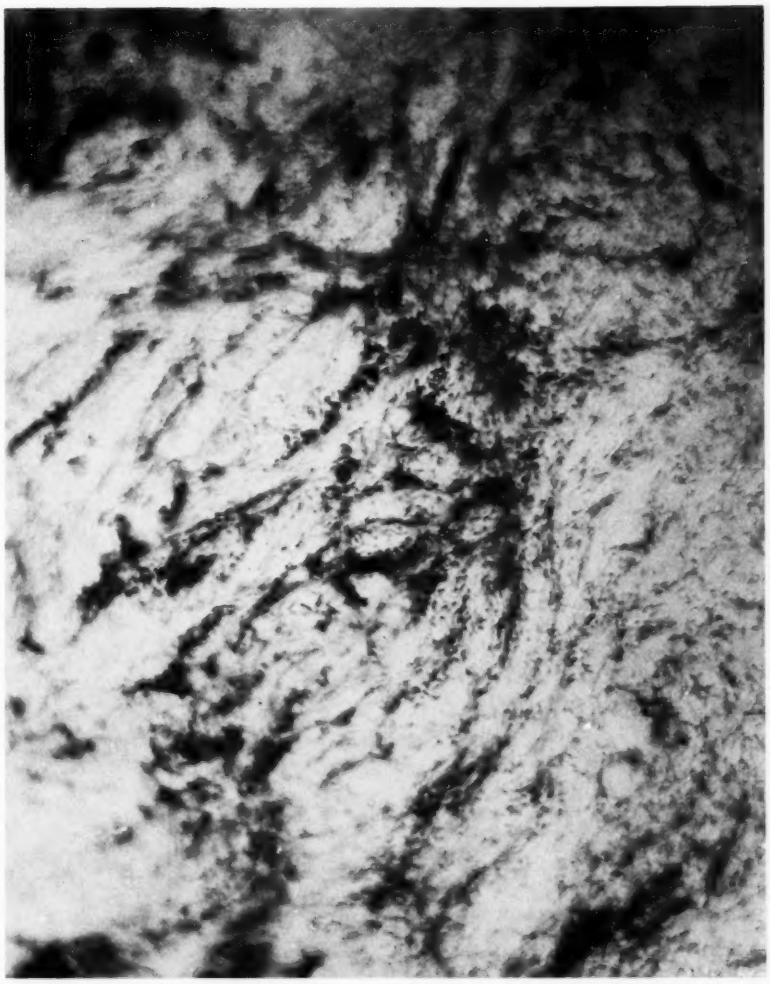

FIG. 3. INFILTRATING DUCT CARCINOMA OF THE BREAST

Vital stain with polychrome toluidin-blue on frozen section. 10 × ocular and 16 mm. objective. Commercial ortho film and K 3 filter with Mazda flood-bulb.

The "placque" and "mass" forms were found with the highest proportions of malignancies, which indicates, not that these gross characteristics are a diagnostic feature, but that in this series clinicians considered those particular clinical findings as the

greatest problem in respect to probable but not certain malignancy, and in respect to choice of operative method.

There were five cases in which the frozen-section diagnosis was reconsidered after the study of the permanent section, in all of which the change of opinion was toward a higher degree of malignancy. There was no case in which the final deliberate opinion arrived at a diagnosis of a lower degree of malignancy than was made at frozen-section. This is a suggestive point, in view of the circumstances under which the usual frozen-section biopsy is made, and will be further discussed below. The changes of diagnosis are discussed in the following table:

CHANGE OF DIAGNOSIS	NUMBER OF CASES	AGES
Non-malignant to malignant	2	35, 42
Malignant to non-malignant	0	
Non-malignant to borderline	2	45, 58
Borderline to definitely malignant	1	44
General non-malignant to specific	0	

It will be seen that there were two cases in which there was a definite reversal of the opinion on malignancy. The other two cases, in which there was a change of opinion from non-malignant to borderline, were probably not quite as radical a reversal of opinion; but for the patient they carried much the same significance. There was only one case in which opinion was changed from borderline to definitely malignant, in which the treatment and clinical prognosis of the patient were affected to a relatively slight degree.

Of this group of cases we have, therefore, 11.1 per cent in which there was an important disagreement in diagnosis between the frozen-section-at-operation examination and the examination of the permanent paraffin section made deliberately and with plenty of time at the pathologist's disposal. In 8.8 per cent of the cases the changes in diagnosis were serious enough to make a radical difference in the patient's treatment and prognosis.

The percentages are not large, and if due to causes beyond human control, could be disregarded in recommending the meth-

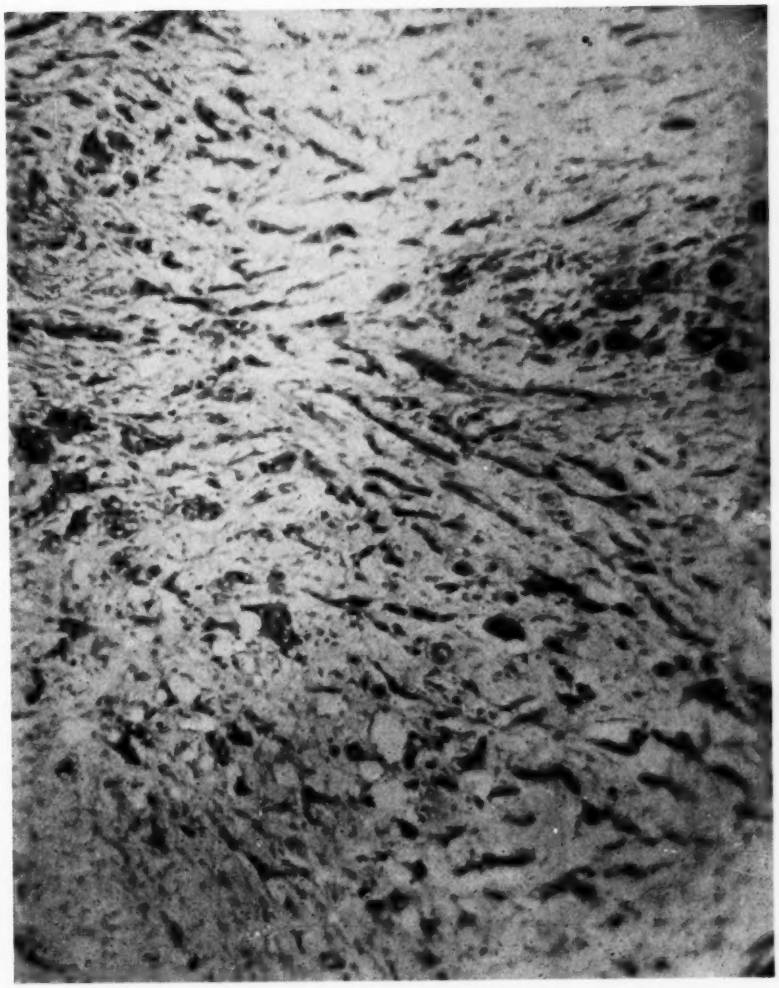

FIG. 4. INFILTRATING DUCT CARCINOMA OF THE BREAST

Hematoxylin-eosin stain on paraffin section. 10 × ocular and 16 mm. objective. Commercial ortho film and K 3 filter with Mazda flood-bulb.

ods under consideration. If, however, this percentage of error is avoidable, there is no excuse for permitting it to continue.

The actual appearance of the frozen-sectioned, vital-stained slide itself and the technical factors involved in its preparation,

have been fairly well eliminated as sources of error in the diagnosis. The *human equation* remains, however.

The frozen-section-at-operation method has a number of serious defects which suffice to nullify any advantage of speed that it may offer. Among these are:

1. There is no record-slide of the procedure to file, as a basis of the pathologist's mental process in making the diagnosis, and to which to refer later in re-studying the problem. This is a most serious defect, as it places the reliance for an important decision on evidence which disappears rapidly and completely, interpreted under circumstances, as will be shown later, highly unfavorable to clear and accurate functioning of the reasoning powers. It is notorious that one of the largest clinics in the country, where the frozen-section method has been brought to a high state of development, can never supply slide records on a case being followed-up elsewhere, though it is always urgent in requesting slides from other clinics or hospitals on patients referred to it.

2. Because of the time limit, sections from only one or two blocks can be examined, whereas it is known that frequently, in doubtful cases, a considerable number of blocks from different parts of the suspected tissue must be taken and sections searched before the final conclusion can be arrived at.

3. In general, it is not always possible to give an opinion on malignancy merely from looking at a slide. Consideration of the patient's complete history and consultation with the clinician are frequently necessary and it would seem that this might be done beforehand in the case of the frozen-section-at-operation method; but the fact remains that it is not being done. The first thing that the pathologist usually knows about a frozen-section-at-operation biopsy is a summons from the surgeon through a subordinate to be present at an arbitrarily fixed time the next morning. The criticism of the frozen-section-at-operation may be in large part a criticism of lack of thoroughness in preoperative study of the patient. An operation for a malignancy is practically never an emergency. Usually the condition has been there for months; a few more days can do no more harm, whereas haste carries with it a large probability of harm.

4. The mere matter of quantitative *time* in studying a section is of the utmost importance. The snapshot method of diagnosing malignancy by a glance at a slide is about as safe and accurate as tossing up a nickel and calling "tails" malignant. There are, of course, cases in which a few seconds' examination will put the diagnosis beyond doubt. But there are too many which are not easy, and in which haste increases the chances for an incorrect diagnosis. Experienced pathologists frequently remark that the longer they look at a section, the more they continue to find in it and the more their findings take shape in their minds. The eye may require fifteen minutes or an hour or several sessions before it picks up details, or the brain as long before it registers them. Psychologists emphasize that *time* is required for the brain to establish connections in the association tracts between the sensory stimulus in hand and the accumulated unconscious or submerged knowledge on the subject.

5. The psychic atmosphere at a frozen-section-at-operation seance is not conducive to accurate scientific reasoning. First, there is the position in which the pathologist himself is placed. To any other consultation, the consultant is asked as to an appointment between peers and his convenience is considered. To one of these occasions, the pathologist is summoned arbitrarily through subordinates; his sensations are those of being subpoenaed. A general attitude prevails about the hospital that any surgical operation is an emergency and is entitled to ride rough-shod over every other consideration in the institution. Though considered less so than the clinician, the pathologist is nevertheless somewhat human. To be unceremoniously called in disregard of previous appointments and other important duties, will, in spite of his own conscientious efforts to be obliging and cooperative, prove distracting to his diagnostic reasoning. It will not require much imagination to comprehend that under such circumstances, patients may suffer from decisions which ought to be made under perfect auspices, and are not.

Then, there is the excitement prevalent among the attendants at these occasions. The entire personnel from operating room to laboratory is highly keyed up long before the operation; technicians and surgical nurses scurry back and forth—and even-

tually someone thinks of notifying the pathologist. The spectacle of a patient lying minute after minute under an anesthetic with nothing being done, surgeons standing in corners in mute dignity with hands folded in towels; everyone else in a flutter—all of this adds to the predicament in the pathologist's mind over the fact that upon his hurried decision may depend either the loss of a life or the unnecessary sacrifice of important tissue. The state of mind thus produced is not favorable to the solution of a complicated problem which requires deliberation, freedom from distraction, and mental poise. On the microscopic slide, there is no "X" marking the spot.

6. It is advisable to add here, in spite of the results of and conclusions from the experimental work above reported in comparing frozen-section, vital-stained slide appearances with those of paraffin and permanently stained and mounted sections, that there is still an element of unfamiliarity in the appearance of the former. If one has seen several hundred vital-stained slides, one may feel confident of understanding them; but they are by no means part of that fundamental, subconscious fabric of knowledge that has been built up by examining and re-examining countless thousands of permanent slides. Arriving at a diagnosis on a doubtful slide is a subtle process involving many factors not apparent to our consciousness, and even this small influence must be considered if our ultimate ideal is perfection.

There are slides on which it is easy to recognize at once frank malignancy or the undoubted absence of it. However, it is becoming established in the minds of students of this subject[1, 2, 3, 4] that in these very cases, the same conclusions are readily arrived at by gross examination of the surgical lesion. Therefore, in these cases, no such flourishing gesture as the frozen section at operation is necessary. *It is in the doubtful cases that the surgeon wants the help of the microscope. And it is in these very doubtful cases that the frozen-section-at-operation method is of no more help in diagnosis and prognosis, than is gross examination*[1, 4]. *The surgeon is no safer, no more certain in basing his procedure on a pathological report made on the basis of this method, than on his own gross pathological judgment.*

FIG. 5. INFILTRATING DUCT CARCINOMA OF THE BREAST

Vital stain with polychrome toluidin-blue on frozen section. 10 × ocular and 4 mm. objective. Commercial ortho film and K 3 filter with Mazda flood-bulb.

In 1935 Ewing[6] wrote, and is quoted in later articles by Simpson[1] and Swift[3]: "Having made more errors by the frozen section in breast cases than by gross examination, I have not resorted to the frozen section field for many years, but rely almost

entirely on gross inspection of breast tissue. Many of my colleagues report to me the same tendency. The cancer surgeon should become highly proficient in the recognition of cancer by sight and touch. No aid from frozen section can replace this capacity."

Bloodgood, who was both a surgeon and a pathologist, was at first enthusiastic about the frozen-section-at-operation method, but by 1931 had completely reversed his attitude[4]. After a restudy of all his cases since 1890, he advises that if there is any doubt in regard to the diagnosis of a tumor at operation, it is safer to remove the tumor with a wide margin of normal tissue and wait the necessary number of days for a careful and deliberate microscopic study of the removed tissue. He emphasizes the fact that the waiting does the patient no harm. "Biopsy for microscopic study is never an operation of urgency," he says.

Simpson[1] advises that when in doubt, the wound should be closed, the patient returned to the room, and the tissue sent to the laboratory for study. In no instance in which this procedure has been carried out in his clinic and carcinoma has been found, has there been a recurrence. "Cancer is not ordinarily a fulminating disease," are his words.

It is the thought of the second operation that may be necessary if malignancy is found, that causes clinicians distress in considering this problem. With competent observers emphasizing that the malignancy is not spread by this procedure if properly done, there remains the reluctance to reopen the wound, and especially to approach the patient with that idea. If the frozen-section-at-operation method were capable of obviating the second operation, there might be some excuse for it. But, it no more prevents it than does gross examination. If sufficient time is taken to prepare and examine proper permanent sections and a second operation is then found necessary, the surgeon can proceed with the consciousness that everything has been done systematically and intelligently. If a frozen-section-at-operation slide is made and the diagnosis later reversed by more careful and deliberate study, the surgeon feels the usual chagrin at having made an error and in approaching the patient

FIG. 6. INFILTRATING DUCT CARCINOMA OF THE BREAST

Hematoxylin-eosin stain on paraffin section. 10 × ocular and 4 mm. objective. Commercial ortho film and K 3 filter with Mazda flood-bulb.

this feeling is most difficult to conceal, if in his own mind he is convinced that the work has not been scientifically carried out.

The writer does not wish to be interpreted as having said that there is no field whatever for the frozen section at operation. It

is the routine abuse of the procedure that is being protested against. It is not being suggested that the method be dropped completely, but that its use be confined to cases where it is of genuine assistance, and that its limitations be recognized. It is being suggested that because there is something of a sensational quality about it, there has been a tendency to ascribe to it, mysterious powers which it does not possess, and to use it under circumstances where the passage of time and the experience of many pathologists has demonstrated that it is capable of doing more harm than good.

Undoubtedly, cases will arise in which the examination of a frozen, vital-stained section during an operation will give information that can be obtained in no other way. *But, it is still contended that the pathologist, not the surgeon, is the man to decide when such a procedure is necessary; and that in the general run of cases a preliminary consultation between the surgeon and the pathologist will either obviate a useless gesture, or put the method into its genuine field of usefulness.*

SUMMARY AND CONCLUSIONS

1. Forty-five cases are reported in which frozen-section-at-operation diagnoses are compared with the diagnoses arrived at by deliberate study of carefully prepared permanent sections. 11.1 per cent of cases were found in which the latter diagnosis radically altered the former.

2. A comparison of the appearances of slides made by the two methods suggests that if any fault for the diagnostic error lies in the slides themselves, it is relatively insignificant in its influence on the final opinion.

3. A discussion is given of modern opinion on the comparative value of the two methods, to the effect that it is probable that the frozen-section-at-operation method is not necessary, that it may do more harm than good, and that deliberate microscopic study of the tissue, even though it may at times necessitate a second operation is in general by far the safer procedure in the interests of the patient.

4. Comments are made on the ethics of pathological consultation.

5. There may be a field in which this method may be of genuine assistance; but preliminary consultation between surgeon and pathologist is the only approach that will assure sound, scientific work.

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BACTERIUM PARATYPHOSUM B MENINGITIS*

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The following case of meningitis due to *Bacterium paratyphosum B* is reported because it affords an opportunity of recording the morphologic pathology of the disease as manifested in the central nervous system.

Meningitis appears to be a rare complication of paratyphoid fever. Lynch and Shelburne[5], in 1931, reported a case of meningitis caused by *Bact. enteritidis*, reviewed the literature on all cases of meningitis due to organisms of the paratyphoid group, and accepted fifteen cases on the basis of "indisputable cultural or serological evidence." Cultures of the spinal fluids of only five of these were positive for *Bact. paratyphosum B*. In addition, two were reported positive for *Bact. paratyphosum B* or *Bact. enteritidis*, while the organisms from another case were not identified. Search of the literature reveals an additional case, reported by Applebaum[2] due to a mixed infection—*Meningococcus and Bacterium paratyphosum B*. Only two cases of *paratyphosum B* meningitis have been reported since publication of the paper by Lynch and Shelburne[5] (Anderson[1], and Jundell[4]). The case herein cited is the fourth reported from the United States.

CASE REPORT

K. P., white, male, was admitted to the Louisville City Hospital in good health on July 9th, 1935, an hour and a half after birth. The infant was sent to the hospital for "routine infant care," merely accompanying its mother who required treatment for post-partum hemorrhage.

The baby weighed 8 lbs. 12 ozs. on admission and progressed normally for

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nine days. On the tenth day it developed mild generalized icterus and slight fever (100°F.), which subsided after twenty-four hours and, although the jaundice persisted, the baby appeared well during the following week.

On the seventeenth day, while still in the hospital, the patient developed bilateral otitis media accompanied by a temperature of 101.2°F. The fever subsided after four hours then slowly returned, registering 103°F. that evening when bulging of the anterior fontanelle, intermittent cyanosis, and wide fluctuations in the pulse and respiratory rate were observed. Ten cc. of turbid, yellowish, purulent spinal fluid were withdrawn which contained Gram-negative rods which could not be identified by direct smear, and 8 cc. of antimeningococcic serum was injected intraspinally as a prophylactic measure. The following day cloudy, xanthochromic fluid was withdrawn by ventricular puncture and cultured. Later that day only a few drops of purulent fluid could be obtained by spinal puncture and but 1 cc. by cisternal puncture. Saline perfused through the cisternal needle could be collected only in minute quantity from the spinal canal in the lumbar region. Small quantities of ten per cent glucose were administered intravenously, but the infant gradually became weaker, the respirations changed to the Cheyne-Stokes type, and ceased on the eleventh day of illness (July 30th). During the last four days of life the temperature remained constant at 104°F. and the pulse rate fluctuated widely.

Laboratory studies. 7–26–35: Spinal fluid-globulin ++++; cell count 234.

7–27–35: Spinal fluid-globulin ++++; cell count 4,307, (98 per cent polymorphs., 2 per cent lymphocytes). Sugar content less than 40 mgm. per 100 cc.

Spinal fluid taken on July 26th showed Gram-negative rods in the direct, or primary smear. Good growth was obtained on plain agar slants; acid and gas were produced in the butt of Russell's double sugar agar slants; acid and gas were produced from glucose, mannitol, and xylose; lactose and sucrose were not fermented. The organisms were actively motile; no indole was produced in tryptophane broth up to 72 hrs. of incubation; and an alkaline reaction was obtained in milk containing bromcresol-purple as an indicator. Spinal fluid specimens taken on July 27th and July 29th yielded Gram-negative rods similar in cultural reactions to those isolated from the spinal fluid taken on July 26th. These cultural reactions are identical with those of the paratyphoid-enteritidis group.

Necropsy report (abbreviated). Necropsy was performed 6½ hours after death. The body was well developed and well nourished. The anterior fontanelle was prominent. The pupils were contracted and equal. The conjunctivae and skin were icteric. There were several lumbar and venipuncture wounds.

The somatic organs showed only several subpleural petechiae; patchy atelectasis, congestion and compensatory emphysema of the lungs; enlargement and pallor of the mesenteric lymph nodes; and congestion of the spleen. There were no intestinal lesions and the Peyer's patches and solitary follicles were not enlarged.

The dura mater was tense and, when incised, about 75 cc. of thick, yellowish purulent fluid oozed out. Its undersurface was granular and covered with fibrinopurulent exudate. The leptomeninges were opaque and granular and bathed in an abundant purulent exudate most marked about the base of the brain, the undersurface of the cerebellum and the poles of the temporal lobes. (Figure 1.)

The brain was large, soft and symmetrical and had broad, flat convolusions. Most of the sulci were narrow and compressed, but the Sylvian and Rolandic fissures were filled with exudate. The lateral ventricles contained purulent

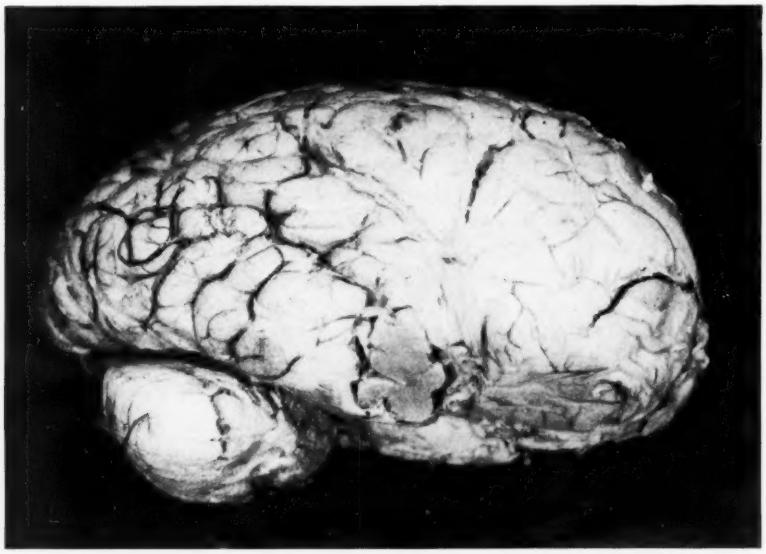

FIG. 1. Photograph of the brain and leptomeninges, reduced two-thirds (lateral view). The exudate is diffuse, but shows localized accumulations which appear as light gray areas obscuring the convolutional markings. The meningeal vessels are intensely congested. The convolutions are edematous and swollen.

fluid showing early organization. They were equal and normal in size. The ependyma was granular. The choroid plexus contained small clots and was covered with purulent exudate. Within the white matter of the cerebrum, near the anterior and posterior horns of the lateral ventricles, were several small areas of recent hemorrhagic necrosis. The pons, peduncles and cerebellum showed no gross lesions.

The basal sinuses contained fluid blood. The middle and internal ears were negative.

The upper portion of the cervical cord was covered with purulent exudate similar to that seen over the surface of the brain.

Anatomical diagnosis. Subacute cerebrospinal meningitis; early purulent encephalitis; thrombosis of the choroid plexus; hyperplasia of the mesenteric lymph nodes; subpleural petechiae; mild, generalized icterus.

FIG. 2. Photomicrograph of the meningeal exudate, magnification 105 diameters. Hematoxylin and eosin stain. The exudate is rich in cells, fibrin, and edema fluid. The blood vessels are prominent.

Bacteriology. Gram-negative rods were isolated from the purulent fluid obtained at autopsy. These organisms gave cultural reactions and characteristics similar to those isolated from the ante-mortem spinal fluid specimens and belonged culturally to the paratyphoid-enteritidis group. This organism was completely agglutinated in a dilution of 1 to 10,240 by New York State *B. paratyphosum B* diagnostic serum (horse) No. 9(1)—titer 1 to 8000. The control tube—saline (0.85 per cent) plus antigen—showed no agglutination on every

occasion. Both culturally and serologically the organism isolated from this case was *Bacterium paratyphosum B*.

Microscopy. The lungs showed only congestion, fetal atelectasis and patchy emphysema. The spleen was intensely congested, its follicles small and widely separated. There was no evidence of a "typhoid reaction." The liver also

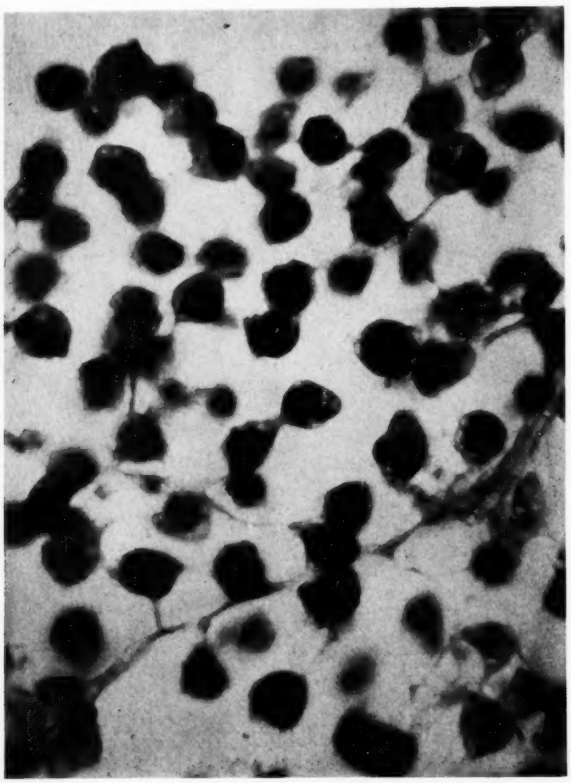

FIG. 3. Photomicrograph of the meningeal exudate, magnification 1280 diameters. Hematoxylin and eosin stain. A majority of the cells are large mononuclears. They have an oval or indented nucleus, often eccentric in position. The vesicular character of the nuclei is not apparent in the photograph. The cytoplasm is irregular in outline and faintly granular.

was congested, its cells pale and swollen. There were no areas of focal necrosis and no bile stasis. The mesenteric lymph nodes showed reticulo-endothelial hyperplasia. In their sinusoids were a few large mononuclear cells, some with phagocytosed red blood cells. The rib and sternal marrow contained an increased number of monocytes and a decrease of normoblasts.

The leptomeninges were extensively infiltrated by a dense inflammatory exudate. The cellular components of the exudate varied considerably. Many of the cells were monocytes. These were from one and one half to twice the size of polymorphonuclears and had a light red, non-granular cytoplasm and an eccentrically placed, large, indented, vesicular nucleus. A large number of

FIG. 4. Photomicrograph of wall of lateral ventricle, magnification 180 diameters. Hematoxylin and eosin stain. Monocytes and leucocytes are accumulated about the smaller vessels. Similar cells are present in the intervening brain tissue.

these were identical with "epithelioid cells" of tuberculous granulation tissue. (Figures 2 and 3.) There were no giant cells, but some of the monocytes contained phagocytosed cell debris. The remainder of the cells in the exudate were neutrophilic polymorphonuclear leucocytes, lymphocytes, and fibroblasts. Some of the neutrophils were pyknotic. There was also a moderate amount of fibrin and albuminous precipitate in the exudate. The small vessels were prom-

inent and contained many white blood cells with a considerable proportion of monocytes. Some of the vessels were so solidly plugged with white cells as to form actual thrombi. These showed infiltration of their walls by monocytes and neutrophilic leucocytes. Several of the large veins also showed a purulent thrombophlebitis.

The brain showed marked edema. In some areas the meningeal inflammation extended into the superficial portions of the cortex and beneath the ependyma of the lateral ventricles. These showed an exudate similar in character to that in the meninges, only less in amount. There was an active inflammatory infiltration about many of the vessels (Figure 4) and, about some of them, small hemorrhages (diapedesis). Some of the sections showed slight glial proliferation and several "rod cells."

The choroid plexus was congested and hemorrhagic (diapedesis). Its surface was diffusely and heavily infiltrated by monocytes and leucocytes, its substance less severely involved. Here there were a few small, discrete granulomatous areas consisting of concentrically arranged, large, pale-staining mononuclear cells. In size and configuration these were identical with the monocytes seen in the meninges. All of these foci were centered about small vessels.

Microscopic diagnosis. Subacute fibrinopurulent meningitis (*Bacterium paratyphosum B*); early purulent encephalitis; hyperplasia of the reticuloendothelium of the thymus and mesenteric lymph nodes; monocytoid reaction of the bone marrow; acute passive congestion of all organs.

COMMENT

There are several interesting features about this case. Morphologically, the granulation tissue in the meninges is very similar to the so-called typhoid reaction seen in the somatic organs in infections caused by organisms of the typhoid-paratyphoid group (Mallory[6]). In the meninges and brain the lesions are rich in monocytes and epithelioid cells. These often occur in focal collections about small vessels many of which contain purulent thrombi or emboli. Occasionally the lesions assume a granulomatous appearance. A categorical diagnosis of paratyphoid meningitis is not permissible solely on the basis of the inflammatory response. However, the granulation tissue is sufficiently specific to warrant a presumptive etiologic diagnosis of typhoid or paratyphoid infection.

Clinically, this case emphasizes the serious prognostic significance of paratyphoid meningitis in children. If we exclude the two cases reported by Caronia & Auricchio[3]—in neither of

which were the spinal fluid findings characteristic of a true meningitis—paratyphoid meningitis is invariably fatal in children aged eleven years or less. Lynch and Shelburne[5] also commented upon this observation.

Epidemiologically, an interesting question arises as to the source of the infection. Absolute proof of ante-natal infection is lacking yet a strong case can be made for regarding this case as an example of intra-uterine infection. The location of the lesions found at necropsy is strongly in favor of an hematogenous rather than an enterogenous infection. There were no intestinal lesions, although they were looked for specifically. The changes in the spleen and liver were not characteristic of typhoid or paratyphoid fever, and the lesions in the mesenteric lymph nodes were minimal in character. The embolic phenomena in the meninges and brain constituted a prominent feature of the inflammation. These, along with the bone marrow changes, are regarded as strongly in favor of an hematogenous infection. Presumptive evidence of a less direct nature is the following: The baby was admitted an hour and a half after birth; it was bottle fed during its entire stay in the hospital; no known cases of paratyphoid fever were treated on any of the wards of the Louisville City Hospital during 1935. Unfortunately, the placenta and umbilical cord were not available for microscopic examination. The mother did not develop symptoms suggestive of paratyphoid fever, but no bacteriological studies were made, so a subclinical infection cannot be excluded.

As an incidental observation, it is worth recording that this patient is the second youngest reported, only the patient of Voight[7] being younger.

SUMMARY AND CONCLUSIONS

1. A case of meningitis due to *Bacterium paratyphosum B* is reported and the poor prognostic import of paratyphoid meningitis in children is emphasized.

2. Paratyphoid meningitis cannot be diagnosed on the basis of the clinical manifestations; the diagnosis rests upon bacteriological examination of the spinal fluid.

3. Microscopically, paratyphoid meningitis is characterized by an exudate rich in monocytes which occasionally form granulomatous foci.

4. Evidence is adduced to suggest that in this case infection occurred during intra-uterine life.

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ISOAGGLUTININ TITERS IN SERUM DISEASE, IN LEUKEMIAS, IN INFECTIOUS MONONUCLEOSIS, AND AFTER BLOOD TRANSFUSIONS*†

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The purpose of this presentation is to report: (a) the finding of very high titers of isoagglutinins in patients with horse serum disease, (b) the finding of very low titers in patients with chronic leukemias, (c) the failure to influence the titer of isoagglutinins by the introduction of large quantities of blood as well as by removal of similar quantities. To provide standards of comparison, the titers of isoagglutinins were studied in a series of 517 persons.

TECHNIC

Determination of the blood group. The blood group of every individual was determined by testing the serum or plasma against known red blood corpuscles of types A and B, and by testing the red corpuscles of the same individual against known serums of types A, B and O. The suspensions of red corpuscles of known type were prepared by adding a drop of blood to 1 cc. of a one per cent solution of sodium citrate in a physiologic solution of sodium chloride. With platinum loops (diameter 3 mm.) two loopfuls of serum or plasma and one loopful of the cell suspension were mixed on a coverslip and suspended over excavated slides. The slides were tilted repeatedly. The results were read with the microscope after standing for thirty minutes at room temperature.

Determination of the titers of isoagglutinins. The serums were inactivated for thirty minutes at 55°C. For the preparation of the red blood cell suspension blood was obtained from several individuals, using one drop of a ten per

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† Since this paper was sent in for publication, G. Bruynoghe (Arch. internat. de Med. Exp. **12:** 397, 1937) reported the finding of high titers of isoagglutinins in a patient of type O and attributed the rise to an injection of horse serum.

cent solution of sodium citrate as anti-coagulant. The blood was stored in the ice box for twenty-four hours before it was used in the preparation of suspensions. Equal quantities of blood of known type from three to five individuals were pooled, washed three times with physiological solution of sodium chloride and used for the preparation of cell suspensions only if there was no trace of hemolysis after the second washing. If hemolysis was present, the cells were discarded. The third centrifugation was continued until the cells were packed to about one-half of the volume of the whole blood and a two per cent suspension with physiological solution of sodium chloride prepared. The cell suspensions were used only on the day they were prepared as the agglutinability of washed cells was found to decrease rapidly, while the cells that were kept in their own plasma maintained their original titer as long as they washed well which was, as a rule, from seven to nine days, which was established repeatedly by setting up daily agglutination tests with pooled serums of known type and titer until the titer of the cells began to drop.

It was on the basis of experience gathered from such repeated experiments that we found it practical and reliable to follow the above procedure.

Serum dilutions beginning with 1:2.5 were prepared in test tubes measuring 75 x 12 mm. One-tenth cubic centimeter of a two per cent suspension of red cells was added to each tube, the tubes were shaken repeatedly, and kept at room temperature for two hours. They were then shaken vigorously until the sediment was suspended and read with the low power of the microscope (32 mm. objective). The titers were recorded in the terms of the final dilutions. The tests were then kept in the ice box over night. On the following morning they were taken from the ice box and kept at room temperature for one hour and again read. The titers were either identical with those of the previous day or they were slightly higher, but never more than by one dilution.

Titrations were repeated frequently to check the reliability of the technic.

DISTRIBUTION OF TITERS IN NORMAL PERSONS AND IN A GROUP OF CHRONIC AMBULATORY PATIENTS

The titers of the isoagglutinins were studied in 517 persons, seventy-eight being healthy young donors for transfusions. The others were mainly ambulatory patients from the out patient department with various chronic ailments. The titers are shown in table 1.

The titers were analyzed separately for each blood group. 1:448 was the highest titer in a very small percentage of cases and the incidence of the lowest titer of 1:3.5 was also fairly rare. The incidence of high anti-A titers was decidedly greater in group B as well as in O than the incidence of high anti-B titers in the groups A and O. The last two columns with the combined anti-B and anti-A titers emphasize the quantitative difference between the two types of isoagglutinins. The difference is reflected in the average titers for each blood

group. The curves (figs. 1, 2, and 3) illustrate graphically the prevalence of higher titers for the isoagglutinin anti-A.

The dilutions are recorded on the horizontal line, while the incidence of the titers is expressed in percentages on the vertical line. Figure 1 represents the titers of the isoagglutinins anti-A in blood group B and of the isoagglutinins anti-B in blood group A. The continuous line of the anti-A agglutinins reaches higher up and farther to the right in the direction of the higher titers than the interrupted line of the anti-B agglutinins.

Figure 2 demonstrates the quantitative relations of the two isoagglutinins in the group O. The prevalence of higher titers for the anti-A agglutinins is very similar as in the two other groups.

TABLE 1

DISTRIBUTION OF TITERS OF ISOAGGLUTININS IN 517 SERUMS

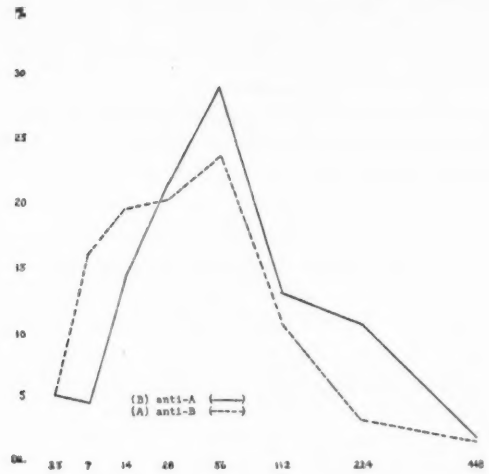
TITERS	(A) ANTI-B		(B) ANTI-A		(O) ANTI-B		(O) ANTI-A		(A) ANTI-B (O) ANTI-B		(B) ANTI-A (O) ANTI-A	
	Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent
1:3.5	13	5.2	8	5.2	2	2.0	1	0.9	15	4.0	9	3.4
1:7	40	15.9	7	4.6	19	17.0	6	5.3	59	16.0	13	5.0
1:14	49	19.5	22	14.4	31	27.0	13	11.5	80	22.0	35	13.1
1:28	51	20.3	33	21.5	33	29.0	29	25.7	84	23.0	62	23.3
1:56	59	23.5	44	28.8	16	14.0	33	29.2	75	21.0	77	28.9
1:112	27	10.8	20	13.1	8	7.0	17	15.0	35	10.0	37	14.0
1:224	8	3.2	16	10.5	4	4.0	11	9.7	12	3.0	27	10.1
1:448	4	1.6	3	1.9			3	2.7	4	1.0	6	2.2
Total.....	251		153		113		113		364		266	
Average titer....	1:50+		1:71+		1:38+		1:76+		1:45+		1:73+	

In figure 3 the isoagglutinins anti-A in the groups B and O were summarized and are represented by the interrupted line while the anti-B agglutinins of groups A and O are represented by the continuous line. The prevalence of the former over the latter is obvious.

The value of the findings in this admittedly small series is enhanced by the circumstance that they agree closely with those of the previous writers on the subject[18, 11, 19, 13, 12, 20].

The evaluation of titers of isoagglutinins requires the consideration of two factors: the agglutinability of the blood corpuscles and the agglutinating property of the serum. Schiff

and Mendlowicz[18] explained the low titers of anti-B agglutinins by the lower agglutinability of the B corpuscles. By testing red corpuscles from different individuals against serums of known

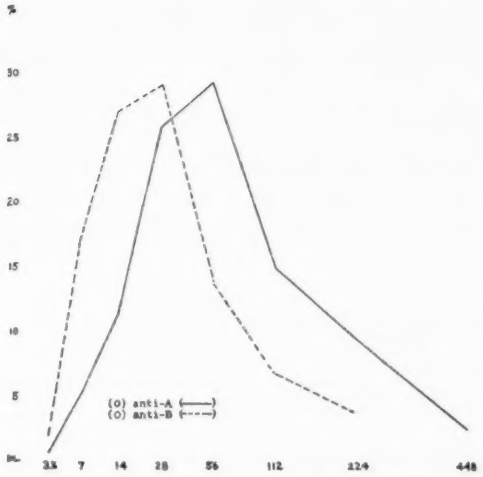


TITERS]	(A) ANTI-B		(B) ANTI-A	
	Number of cases	Per cent	Number of cases	Per cent
1:3.5	13	5.2	8	5.2
1:7	40	15.9	7	4.6
1:14	49	19.5	22	14.4
1:28	51	20.3	33	21.5
1:56	59	23.5	44	28.8
1:112	27	10.8	20	13.1
1:224	8	3.2	16	10.5
1:448	4	1.6	3	1.9
Total.....	251		153	
Average titer....	1:50+		1:71+	

FIG. 1

agglutinating titer, Schiff and Huebener[17] found that the sensitivity of A corpuscles varied from 1:25 to 1:800, and that the sensitivity of B corpuscles was lower ranging from 1:24 to 1:400.

Thomsen and Kettel[19] observed a very low degree of agglutinability of the red corpuscles in the newborn and infant and a gradual rise until the maximum agglutinability was reached

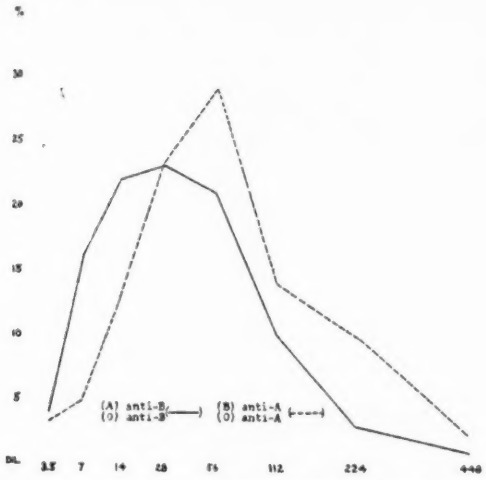


TITERS	(o) ANTI-B		(o) ANTI-A	
	Number of cases	Per cent	Number of cases	Per cent
1:3.5	2	2.0	1	0.9
1:7	19	17.0	6	5.3
1:14	31	27.0	13	11.5
1:28	33	29.0	29	25.7
1:56	16	14.0	33	29.2
1:112	8	7.0	17	15.0
1:224	4	4.0	11	9.7
1:448			3	2.7
Total.....	113		113	
Average titer....	1:38+		1:76+	

FIG. 2

between the years from sixteen to thirty. After the peak of sensitivity was reached, it retained its full strength to the end of life. It was found unimpaired even in individuals 100 years

old. In two hundred individuals from thirty to forty years old, the red corpuscles of eighty-five per cent were agglutinated in a dilution of 1:256, five per cent in the next higher dilution of



TITERS	(A) ANTI-B (O) ANTI-B		(B) ANTI-A (O) ANTI-A	
	Number of cases	Per cent	Number of cases	Per cent
1:3.5	15	4.0	9	3.4
1:7	59	16.0	13	5.0
1:14	80	22.0	35	13.1
1:28	84	23.0	62	23.3
1:56	75	21.0	77	28.9
1:112	35	10.0	37	14.0
1:224	12	3.0	27	10.1
1:448	4	1.0	6	2.2
Total.....	364		266	
Average titer....	1:45+		1:73+	

FIG. 3

1:512, five per cent in the next lower dilution of 1:128, and only five per cent were agglutinated at still lower dilutions. They conclude that the agglutinating properties of the serums are

responsible for the titer of the isoagglutinins to a greater extent than was assumed by Schiff and Mendlowicz.

Attention has been called in recent years to the danger of so-called universal donors with high titers of isoagglutinins. It would appear from my study that the danger would be greater when type O donors are used for type A patients than for type B.

THE RELATION OF THE TITERS OF ISOAGGLUTININS TO AGE

Schiff and Mendlowicz[18], Thomsen and Kettel[19] and several other investigators after them, observed a relation between the titers of isoagglutinins and the age of the person. At birth the

TABLE 2

THE TITERS OF ISOAGGLUTININS IN THE DIFFERENT AGE GROUPS

AGE	(A) ANTI-B		(B) ANTI-A		(O) ANTI-B		(O) ANTI-A		(A) ANTI-B (O) ANTI-B		(B) ANTI-A (O) ANTI-A	
	Number of cases	Average titers	Number of cases	Average titers	Number of cases	Average titers	Number of cases	Average titers	Number of cases	Average titers	Number of cases	Average titers
1–20	26	1:42+	11	1:42+	14	1:34+	14	1:66+	40	1:39+	25	1:54+
21–30	49	1:44+	27	*1:110*+	34	*1:48*+	34	*1:101*+	82	1:46+	61	*1:105*+
31–40	45	*1:76*+	34	1:75+	33	1:34+	33	1:77+	78	*1:58*+	67	1:76+
41–50	55	1:55+	26	1:53+	18	1:23+	18	1:50+	73	1:47+	44	1:51+
51–60	30	1:32+	24	1:53+	7	1:18+	7	1:25+	37	1:30+	31	1:47+
61–70	37	1:41+	22	1:50+	4	1:15+	4	1:35+	41	1:39+	26	1:47+
71–80	9	1:19+	6	1:31+	1	1:14+	1	1:28+	10	1:19+	7	1:31+

isoagglutinins are either absent or of a very low titer. They increase gradually during the first years of life. The highest titers are more common in youth and they tend to drop with advancing age. Thomsen and Kettel studied the largest series of 1,500 persons. The highest titers were present in the age of five to ten years.

Table 2 shows the average titers in the different age groups of a series of 512 persons, of whom 251 were of group A, 150 of group B and 111 of group O. The highest titers were in the decade twenty-one to thirty in the groups B and O, and in the decade thirty-one to forty in the group A. The drop with the

advancing age is very striking and confirms the results of the previous writers. The relation between the age and the titer is not constant and absolute in any individual case. Very low titers were observed in young individuals and high titers in old persons, but in general the correlation is quite definite.

DISTRIBUTION OF THE ISOAGGLUTININ TITERS ACCORDING TO SEXES

There were 252 males and 265 females. The average titers for the isoagglutinins (A) anti-B were 1:56+ for women and 1:41+ for men, for the agglutinins (B) anti-A they were 1:73+ for women and 1:68+ for men, for the agglutinins (O) anti-B they were 1:35+ for women and 1:38+ for men and for the agglutinins (O) anti-A the titers were 1:64+ for women and 1:82+ for men. The results show higher titers for the (A) anti-B agglutinins in women and for the (O) anti-A agglutinins in men. These findings are recorded here without any further conclusions.

EFFECT OF THE INTRODUCTION OF COMPATIBLE BLOOD UPON THE TITERS OF ISOAGGLUTININS

I studied the effect of the introduction of blood upon the titer of isoagglutinins in seventy-two blood transfusions. Blood was obtained before the transfusions from the recipient as well as from the donor, and again from the recipient immediately after the blood transfusion. In as many cases as it was possible, it was obtained from the recipients after varying intervals following the transfusion. The intervals varied from twenty-four hours to ten days. Fourteen patients received two transfusions in intervals from one to fourteen days, four received three transfusions in intervals from one to twenty-three days, one received four transfusions in intervals of two, four, and eight days, and one received ten transfusions every forty-eight hours in the course of twenty days. In all these cases the blood was obtained from the recipient as well as from the donor before and after each transfusion. The quantities of transfused blood were 150 cc. in three cases, 200 cc. in eight cases, 250 cc. in seven cases 300 cc. in six cases, 350 cc. in eight cases, 400 cc. in ten cases, 425 cc. in one case, 450 cc. in one case, 475 cc. in two cases, 500

cc. in twenty-eight cases, 520 cc. in one case and 700 cc. in one case. The patient given ten transfusions in twenty days received altogether 2,050 cc. of blood.

Forty-one of the patients belonged to blood group A; twenty-nine of them received blood from donors with the identical group and twelve from donors with group O. Eight of the recipients had the group B; five of them were transfused with blood of the same group and three with blood of group O. Twenty-nine recipients belonged to group O.

The titers of isoagglutinins were determined in each case simultaneously in the specimens of blood obtained from the recipients and donors before and after each transfusion, using the same suspension of blood cells, and in the cases of repeated blood transfusions the titrations were repeated after the blood was obtained from the last transfusion; in sixty-seven transfusions there was either no change in the titers of the agglutinins or the difference was only one dilution which I considered to be within the range of technical variability. After the transfusion there was in three instances a drop in the titers amounting to two dilutions (twice from 1:448 to 1:112, once from 1:56 to 1:14), and in two instances similar rises were noted. The explanation for that was sought in the differences in titers between the donors and the recipients, in the quantities of injected blood, but no such relation could be detected because the same factors were present in many of the other transfusions without the same effect.

The titers of the isoagglutinins in the recipients as well as in the donors were within the range of normal variation, except two excessively high titers in recipients. In some instances the rise or the drop of the titers in the specimens obtained after the transfusion were parallel to the higher or lower titers in the blood of the donors. However, at least as many instances were found where similar differences in the titers of the agglutinins in the blood of the donors did not influence the titers in the blood of the recipients.

It seems that transfusions of blood do not influence materially the titers of the isoagglutinins in the recipients.

In eight donors the titers of isoagglutinins were studied before and after removal of varying quantities of blood. No change was noted.

VARIATION OF TITERS OF ISOAGGLUTININS IN DISEASE

While it is generally accepted that there is no relation between the characteristic blood group factors in the red corpuscles and diseases, the quantitative variations in the titers of isoagglutinins in the serums of different individuals suggested a search for a relation between these variations and diseases. Repeated tests in the same individuals over extended periods have shown that in health the titer of isoagglutinins tends to maintain a constant level. Lehman[13] tested his own anti-A and anti-B agglutinins daily for one month and at longer intervals for one year and noticed no change. Albertsen[1] found a similar constancy in several individuals during a period of over one year. Variations of titers have been reported in a great many diseases. Most of the reports deal with isolated instances and are not very convincing. I shall quote merely a few examples. Albertsen[1] noted an elevated titer in a case of biliary cirrhosis and a drop in sepsis. Minkewitsch and Raskin[14] observed variations in severe infections (typhoid and typhus fever, miliary tuberculosis and pneumonia). He believed that they indicated changes in resistance. Hoche[8] found markedly elevated titers in fifteen patients with hyperthyroidism, while no elevation was present in ten patients with goiter but without signs of hyperthyroidism. The high titers persisted in a few patients who were seen long after a successful thyroidectomy cured the condition. There was no relation between the titer of the isoagglutinins and the basal metabolic rate.

Schiff and Mendlowicz[18] were the first to record the frequency of very low titers of isoagglutinins in thirty-one cases of leukemias, although some of the cases had fairly high titers. Other writers observed before Schiff and Mendlowicz the failure of leukemic patients to respond with the production of agglutinins to injections of typhoid vaccines[15,16,10], and of other microorganisms. In two instances leukemic patients did not develop agglutinins when they contracted typhoid fever and paratyphoid

fever. Bernstein[2] reported recently the finding of low titers of heterophilic agglutinins against sheep cells in leukemia. Zuendel[20] corroborated the observations of Schiff and Mendlowicz in a study of twelve patients with leukemia. He noted that the titers were somewhat higher in irradiated and improved cases.

I studied thirty-eight patients with leukemia, among them eighteen with acute leukemia, thirteen with chronic leukemic lymphadenosis, five with chronic leukemic myelosis, one with chronic aleukemic myelosis and one with monocytic leukemia.

TABLE 3

TITERS OF ISOAGGLUTININS IN PATIENTS WITH SERUM DISEASE, IN PATIENTS TREATED WITH HORSE SERUM BUT WITHOUT SERUM DISEASE, IN LEUKEMIAS AND IN INFECTIOUS MONONUCLEOSIS

DIAGNOSIS	(A) ANTI-B		(B) ANTI-A		(O) ANTI-B		(O) ANTI-A		(A) ANTI-B (O) ANTI-B		(B) ANTI-A (O) ANTI-A		TOTAL NUMBER OF CASES
	Number of cases	Average titers	Number of cases	Average titers	Number of cases	Average titers	Number of cases	Average titers	Number of cases	Average titers	Number of cases	Average titers	
Normal cases	251	1:50+	153	1:71+	113	1:38+	113	1:76+	364	1:45+	266	1:73+	517
Serum disease	11	1:769+	3	1:187+	19	1:543+	19	1:1001+	30	1:630+	22	1:856+	33
Horse serum treated patients without serum disease	5	1:74+	1	1:14+	9	1:154+	9	1:190+	14	1:125+	10	1:172+	15
Acute leukemia	7	1:37+	2	1:60+	9	1:18+	9	1:51+	16	1:26+	9	1:63+	18
Chronic leukemia	4	1:15+	4	1:4+	8	1:13+	8	1:20+	12	1:13+	12	1:20+	16
Chronic leukemia treated with x-ray	1	1:56+			3	1:57+	3	1:103+	4	1:57+			4
Infectious mononucleosis	20	1:58+	8	1:72+	16	1:32+	16	1:65+	36	1:46+	24	1:67+	44

The titers of the isoagglutinins in the majority of the cases of chronic leukemias were considerably below the average titers in non-leukemic patients of the same blood groups and ages. There was no difference between the lymphatic and myelogenous forms of the disease. Table 3 records the average titers in the chronic leukemias and in the normal controls. In acute leukemias the titers were considerably higher almost approaching the normal. I observed normal or even slightly elevated titers in patients with chronic leukemias who were exposed to intensive x-ray therapy.

The possible influence of x-ray therapy had been considered by

previous writers. According to Zuendel[20] irradiation elevated the titers of isoagglutinins in leukemic patients, on the other

TABLE 4

CHRONIC LEUKEMIAS

NUMBER	DIAGNOSIS	AGE	(A) ANTI-B	(B) ANTI-A	(O) ANTI-B	(O) ANTI-A
42	Myelosis	4		1:3.5+		
27	Monocytic leukemia	30			1:28+	1:28+
21	Lymphadenosis	40			1:7+	1:28+
22	Myelosis*	40			1:3.5+	1:56+
44	Aleukemic myelosis	43			1:14+	1:14+
32	Myelosis	44	1:3.5+			
17	Lymphadenosis	45			1:7+	1:14+
14	Lymphadenosis*	45			1:56+	1:224+
24	Lymphadenosis	50			1:7+	1:3.5+
4	Lymphadenosis*	51	1:56+			
37	Myelosis*	52			1:112+	1:28+
1	Lymphadenosis	62		1:3.5+		
2	Lymphadenosis	62			1:28+	1:56+
3	Lymphadenosis	64			1:7+	1:14+
36	Lymphadenosis	64	1:14+			
33	Lymphadenosis	66		1:7+		
38	Lymphadenosis	67	1:28+			
40	Lymphadenosis	67			1:3.5+	1:3.5+
20	Myelosis	68		1:3.5+		
43	Lymphadenosis	70	1:14+			

Average Titers of Normal Cases of the Above Age Group

AGE	(A) ANTI-B	(B) ANTI-A	(O) ANTI-B	(O) ANTI-A
1–20	1:42+	1:42+	1:34+	1:66+
30–40	1:76+	1:75+	1:34+	1:77+
41–50	1:55+	1:53+	1:23+	1:50+
51–60	1:32+	1:53+	1:18+	1:25+
61–70	1:41+	1:50+	1:15+	1:35+

* Treated with x-ray.

hand, Hoche and Moritsch[9] and Gruenwald[7] observed no effect of that kind in non-leukemic irradiated patients.

Most of my patients with chronic leukemias were older than forty. To show that the low titers were not merely an attribute of the advanced age of the patients, table 4 lists the ages and

titers and at the bottom the average titers for each blood and age group of non-leukemic patients.

It is possible that the normal or even elevated titers in leukemia in the reports of Schiff and Mendlowicz and Zuendel were due to the presence of cases with acute leukemia or to effects of x-ray therapy.

I observed a very marked elevation of the isoagglutinin titers in thirty-three patients who developed serum disease following

TABLE 5

TITER	TITERS OF ISOAGGLUTININS IN THE SERUM OF PATIENTS WITH HORSE SERUM DISEASE						TITERS OF ISOAGGLUTININS IN THE SERUM OF PATIENTS TREATED WITH HORSE SERUM BUT WITHOUT SERUM DISEASE					
	(A) anti-B	(B) anti-A	(O) anti-B	(O) anti-A	(A) anti-B (O) anti-B	(B) anti-A (O) anti-A	(A) anti-B	(B) anti-A	(O) anti-B	(O) anti-A	(A) anti-B (O) anti-B	(B) anti-A (O) anti-A
1:3.5									1		1	
1:7	1				1		1				1	
1:14				1		1		1	1		1	1
1:28	2		3	1	5	1	1		1	1	2	1
1:56	2		3	5	5	5	2		2	4	4	4
1:112	2	1	4	5	6	6			3	1	3	1
1:224	2	2	5	3	7	5	1			2	1	2
1:448	1		3	1	4	1						—
1:896				1		1			1	1	1	1
1:792				1		1						
1:7168	1		1		2							
1:14336				1		1						
Total.....	11	3	19	19	30	22	5	1	9	9	14	10
Average titer.....	1:769	1:187	1:543	1:1001	1:630	1:856	1:74	1:14	1:154	1:190	1:125	1:172

therapeutic injections of horse serum or of horse immune serums (table 3). The rise was most marked in the (A) anti-B agglutinins, where the average titer was fifteen times that of the corresponding normal average; it was least in the (B) anti-A agglutinins, but that may be due to the very small number of cases in that group. Contrary to the findings in serum disease, patients treated with horse serum or with horse immune serums who did not develop serum disease showed only slight elevations of isoagglutinin titers.

Table 5 lists all the titers in horse serum treated patients because average figures alone do not always give a true picture. The greater frequency of high titers in patients with serum disease as compared with those listed on the right half of the table is obvious. On the other hand low titers may be seen in patients with serum disease, although in this series about two-thirds of the patients with serum disease had high normal or markedly elevated titers.

Table 6 lists the titers of isoagglutinins in five patients before the injection of horse serum, at intervals after the injection and their relation to the time of onset of serum disease. In several instances, it could be recorded that the rise preceded the onset of symptoms of serum disease and it became particularly elevated after serum disease set in. Case No. 32 is included to show that considerable elevations of the agglutinating titer occur even without serum disease.

I could find only a few references in the literature to the effect of injections of horse serum upon the titers of isoagglutinins, all recording observations contrary to mine. It seems that my observations are the first to record frequent and marked elevations of the titers of isoagglutinins following injections of horse serum. Minkewitsch and Raskin[14] remark that there was no change in the titer of human isoagglutinins after inoculations of toxin and antitoxin mixtures. Gelli and Tarozzi[6] found a drop in the titer during serum disease in twenty children treated with diphtheria antitoxin. It is difficult to evaluate the titers in Gelli and Tarozzi's publication because the data about the technic are very incomplete. Friedemann and Beer[5] found no significant elevations of the titers of isoagglutinins following injection of horse serum.

The elevation of the isoagglutinins following injections of horse serum and particularly in serum disease is apparently a part of a general rise of various normal antibodies and especially of anti-sheep agglutinins that was described before[3].

It is well known how difficult it is at times to secure isoagglutinating serums with high titers for the purpose of blood grouping. To meet that difficulty it may prove practical to collect blood from patients with horse serum disease.

TABLE 6

INCREASE OF ISOAGGLUTININS AFTER INJECTIONS OF HORSE SERUM AND PARTICULARLY IN SERUM DISEASE

NUMBER	TYPE		TITER
27	A	Before injection	1:7+
		5 days after injection	1:448+
		7 days after injection*	1:1792+
		9 days after injection	1:7168+
		47 days after injection	1:224+
28	A	Before injection	1:14+
		2 days after injection	1:28+
		7 days after injection	1:112+
		11 days after injection*	1:448+
		15 days after injection	1:448+
		16 days after injection	1:224+
		42 days after injection	1:112+
33	A	Before injection	1:3.5+
		7 days after injection*	1:56+
		14 days after injection	1:224+
46	B	Before injection	1:7+
		5 days after injection	1:28+
		10 days after injection*	1:224+
		14 days after injection	1:224+
		21 days after injection	1:56+
32†	A	Before injection	1:28+
		10 days after injection	1:28+
		14 days after injection	1:224+
		21 days after injection	1:224+

NUMBER	TYPE		ANTI-A	ANTI-B
53	0	Before injection	1:28+	1:7+
		7 days after injection	1:7+	1:7+
		14 days after injection*	1:112+	1:112+
		28 days after injection	1:56+	1:112+
		42 days after injection	1:112+	1:224+
		49 days after injection	1:112+	1:112+

* Onset of serum disease.

† This patient did not develop serum disease.

In connection with the above observations in serum disease, I wish to record that in forty-four patients with infectious mononucleosis the titers of isoagglutinins were within the normal range (table 3). That is of some interest because the titer of agglutinins against sheep red corpuscles is very high in infectious mononucleosis and usually considerably higher than in serum disease. The comparison of the differences in the isoagglutinin titers in serum disease and in infectious mononucleosis adds more evidence to the already established difference in the nature of the antibody response in the two conditions[4].

SUMMARY AND CONCLUSIONS

1. A study of the distribution of titers among 517 normal persons and chronic ambulatory patients confirmed the previously reported greater frequency of higher titers for the anti-A isoagglutinins in blood groups B and O, as compared with the anti-B isoagglutinins in blood groups A and O.

2. That would indicate that there is a relatively greater danger when the so-called universal donors with high titers of isoagglutinins are used for type A patients than for patients of type B.

3. It was confirmed that high isoagglutinin titers are most common among young persons, becoming less frequent with advancing age.

4. The distribution of the titers was quite similar in both sexes.

5. The prevalence of low titers of isoagglutinins in leukemias was confirmed, but only in the chronic form of the disease, particularly in cases that did not receive x-ray therapy. Most cases of acute leukemia and patients with chronic leukemia who received intensive x-ray therapy showed normal or even slightly elevated titers.

6. Very high titers were observed in many patients with serum disease. It is suggested that patients with serum disease may supply serums with high titers for blood grouping tests.

7. In infectious mononucleosis the titers of isoagglutinins were within the range of normal variations.

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RELATIONS OF THE ADRENAL GLANDS AT AUTOPSY WITH CLINICO-PATHOLOGICAL FINDINGS AND WITH BLOOD VITAMIN C*

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According to all standard textbooks on pathology, central cavitation of the adrenal glands found at autopsy, is indicative of post mortem degeneration. Naturally this conclusion excludes as an artefact the mechanical or artificial splitting within the medulla which is usually the result of manipulation by the autopsy technician in removing or handling the organs. The cavitation referred to, and which we studied, is the central autolysis of the organ and usually includes the entire medulla and inner portion of the cortex.

The implication given by the textbooks is that central autolysis of the adrenal glands will be absent in those cadavers which are well preserved, and present in those cadavers which show some degree of post mortem degeneration in the remainder of the body. Our experience has not corroborated this conclusion, however, for we have observed the phenomenon to be present in well preserved bodies, and, conversely, we have observed the phenomenon to be absent in bodies whose abdominal walls showed marked post mortem degeneration. Furthermore, in a review of all autopsies (122) performed by one of us (W. F.) in one calendar year, no relation was found to exist between the incidence of central cavitation of the adrenal glands and the length of time that had elapsed from death to examination

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(Figure 1); all cadavers had been subjected to the same refrigerating system. In short, in our experience the occurrence of central autolysis of the adrenal glands has not been consistent

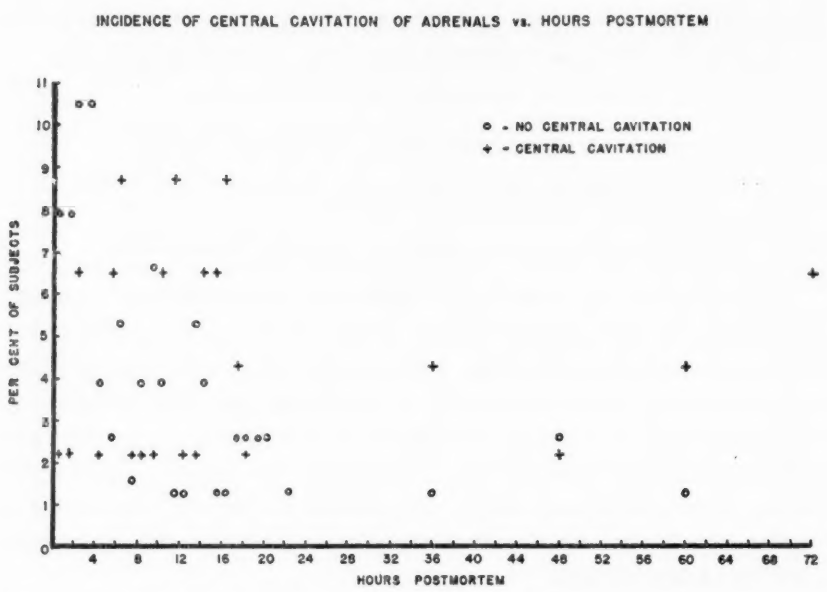

FIG. 1. A DISTRIBUTION GRAPH DEPICTING THE INCIDENCE OF CENTRAL CAVITATION OF THE ADRENAL GLANDS IN RELATION TO THE LENGTH OF TIME BETWEEN DEATH AND THE POSTMORTEM EXAMINATION IN 122 CONSECUTIVE CASES

Although there is a suggestion that the shorter the time postmortem, the less chance there may be of finding central cavities in the adrenal glands, there is yet no absolute relationship. Note that more than 11 per cent of the subjects were found to have central cavities even though they were autopsied within three hours after death.

with post mortem degeneration of the remainder of the body; hence the phenomenon seemed to demand more careful study.

METHOD

Various factors, at one time or another suspected of being dependent on the function of the suprarenals in vivo, were studied in correlation with the postmortem findings of the glands. The patient's age, sex, cause of death, psychi-

atric diagnosis,* length of residence in the institution, systolic blood pressure at rest, pulse pressure at rest, terminal febrile state,† nutritional index,‡ fasting blood morphological values,§ and fasting blood chemical values¶ were studied in turn, but no relation was discovered between any of these factors and the incidence of central cavitation.

Since the suprarenal glands have been found to be important storage depots of ascorbic acid, it was thought that the existence of central cavities at autopsy might be allied to the general ascorbic acid content of the living body. The circulating blood was the logical point at which to investigate this aspect, and the following study was thus undertaken, using the method of Farmer and Abt[1] to determine the amount of ascorbic acid, or in other words, the vitamin C level. While this method evaluates the acid in its reduced form, it has been shown that the total ascorbic acid content of the blood varies in direct proportion to the reduced fraction; and therefore our objective was not altered.

The morning after a patient was placed on the "Danger List," the reduced ascorbic acid content of his blood plasma was determined. When the patient died, an autopsy was performed as soon as permission was granted. In case the patient lived and was still dangerously ill at the end of a week, the determination was repeated. This procedure was continued until the patient had either recovered or died. In this way there was obtained a fair estimate of the reduced ascorbic acid level of the patient's blood plasma during the week before his death.

The method used to determine the reduced ascorbic acid of the blood plasma followed closely that published by Farmer and Abt. All blood specimens were obtained before the patients had had breakfast, or 15 hours or more after the preceding meal. A mixture of potassium oxalate and sodium cyanide (10 parts of potassium oxalate and 3 parts of sodium cyanide) was used as an anticoagu-

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* Taken from official hospital records. The diagnoses are arrived at by the majority vote of all attending psychiatrists.

† All temperature were rectal. Febrile state: temperature over 102°F. just prior to death, or temperature maintained between 101° and 102°F. for three or more days just prior to death. Subnormal temperature: 98°F. or lower.

‡ Calculated from the Metropolitan Life Insurance Table of normal averages. Takes into account the individual's age and height.

§ Values studied: hematocrit, hemoglobin (Haden-Hauser), erythrocyte and leucocyte counts, and neutrophile percentage. The blood samples were taken before breakfast while the patient was in a post-absorptive state. The values were determined and recorded by the same individual, L. DeL.

¶ Values studied: total non-protein nitrogen, sugar, and chlorides. The blood specimens were taken before breakfast, while the patient was in a post-absorptive state. The values were determined and recorded by the same individual, A. I. W.

lant. The plasma proteins were precipitated by a metaphosphoric acid mixture within an hour after the blood specimen was obtained; the time element in this step is important, inasmuch as ascorbic acid readily undergoes auto-oxidation. The vitamin C determinations were made and recorded by the same individual, R. S.

After death all bodies were subjected to the same preparatory routine for the undertaker. Since this procedure takes from one-half to one full hour, and sometimes longer, the bodies remained at least 30 minutes at room temperature before they arrive in the morgue. Unless the body was autopsied immediately, it was placed in an electrically refrigerated box and the cooling process was consequently the same for all bodies.

The bodies were autopsied by the pathologist and the technique of removing the adrenals was the same in all instances. The glands were carefully dissected from the kidneys and fat with a sharp scalpel and the excess fat was trimmed off with sharp scissors, the organs being held with forceps. Serial cross sections were made with a sharp sectioning knife, immediately after the glands were weighed. The presence or absence of autolysis in the central portion of the organs was noted. As an additional qualitative test, the sections were then immersed in a 10 per cent neutral silver nitrate solution for at least 30 minutes. (Silver nitrate precipitates black metallic silver oxide on tissue containing vitamin C, Hamilton[2].)

RESULTS

As was stated previously, in a study of 122 consecutive cases no relation was found between the incidence of central cavitation of the adrenal glands at autopsy and the patient's age, sex, cause of death, psychiatric diagnosis, length of residence in the institution, basal systolic blood pressure, basal pulse pressure, terminal febrile state, nutritional index, fasting blood morphological values, or fasting blood chemical values. Because of the absence of any correlation, the details of the studies are omitted; suffice it to say that all the data were subjected to statistical analysis by E. M. Jellinek, Head of the Biometrical Department of the Worcester State Hospital.

The results of the correlation study between the incidence of central cavities in a patient's adrenal glands at post mortem, and the fasting reduced ascorbic acid value of his blood plasma in vivo, are shown in Figure 2. A total of 42 observations are recorded.

A few of the patients were autopsied within an hour after

death and some were examined two days, three days, or even longer, after death. There was an even distribution between men and women. The ages varied from 42 to 84 years, and the majority ranged beyond 60 years.

The distribution of the cases is most interesting for its well-defined groupings, both in respect to themselves and in respect

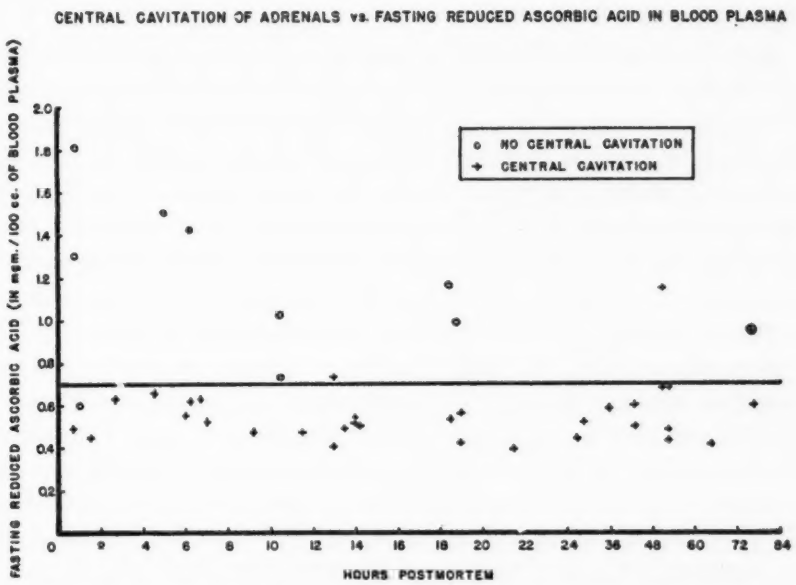

FIG. 2. A DISTRIBUTION GRAPH DEPICTING THE INCIDENCE OF CENTRAL CAVITATION OF THE ADRENAL GLANDS IN RELATION TO THE ASCORBIC ACID LEVEL OF THE BLOOD PLASMA IN VIVO AND THE LENGTH OF TIME WHICH HAS ELAPSED FROM DEATH TO THE POSTMORTEM EXAMINATION

Forty-two consecutive cases were studied

to the ante-mortem vitamin C content of the blood plasma. It is easily evident, even from a rapid glance at the figure, that the cases presenting no central cavities in the adrenal glands at autopsy segregate themselves *above* a certain reduced ascorbic acid value, and the cases which did present central cavities in the adrenal glands at autopsy segregate themselves *below* that value. In this connection it is worth noting that Farmer and Abt sug-

gest in their paper that if the reduced ascorbic acid of the blood plasma be less than 0.7 mgm. per cent, clinical signs of scurvy will appear. As their conclusion is by no means proven as yet, this level must, for the time being, be considered as arbitrary. (Nevertheless, in this study 32 of the cases had ascorbic acid values below 0.7 mgm. per cent. That such a large percentage of our patients have a low level is the subject of a forthcoming publication.)

But it is remarkable that, except in a single instance (in which the patient was autopsied within one hour after death) all those who had an ante-mortem fasting reduced ascorbic acid value of less than 0.7 mgm. per cent also had central autolysis in both adrenal glands at autopsy. Also in these cases no black color change was produced on the surfaces of the sectioned glands even after one hour's immersion in a 10 per cent neutral silver nitrate solution. On the other hand, with the exception of three cases, all those having an ante-mortem fasting reduced ascorbic acid value greater than 0.7 mgm. per cent had no central autolysis in either adrenal gland. The immersed sections of these glands in a 10 per cent neutral silver nitrate solution showed blackening of the tissue surfaces in a very few minutes. Another noteworthy point is that the medullae of the sections turned black many minutes before the cortices. It may be that the medulla contains ascorbic acid in a more readily available form than the cortex, so that in the secretion of the acid from the glands the medullae lose it first. This explanation is substantiated by the fact that the various forms of ascorbic acid in the body differ as to their lability, the reduced form being more labile than the combined as has been shown by Farmer and Abt. This being so, it is logical to assume that that part of the organ reacting to the reagent first contains the more labile, or in other words, the more available form; in this case it is the medulla. It is also logical to assume that in the ascorbic acid depletion of the adrenal glands the more available form would be lost first; and consequently in times of need, it would be the medulla that is first depleted.

The exceptions in the above group bear some explanation.

The patient who had a vitamin C value of 1.15 mgm. per cent, and yet had central cavities in both glands at autopsy, died of a pneumococcic pneumonia, empyema, and septicemia. Her terminal rectal temperature was 105°F., and the body remained at room temperature for more than three hours before refrigeration. The two foregoing circumstances are to be noted, since it is a fact that ascorbic acid undergoes auto-oxidation very rapidly in warm temperatures. As a result it is reasonable to suppose that a considerable amount of ascorbic acid was decomposed in this patient after death. If, as seems to be brought out by this study, an adequate bodily content of ascorbic acid is related to intact adrenal glands at autopsy, it is probable that in this case the presence of central autolysis 50 hours after death was due to slow refrigeration, and that the finding prognosticated by the ante-mortem reduced ascorbic acid value could not be expected to obtain.

The body of the patient whose vitamin C value was 0.95 mgm. per cent was not placed in the morgue until two hours after death; her terminal temperature, however, had been normal. At post mortem 76 hours later, one adrenal gland was intact and gave a positive silver nitrate test, and the other adrenal gland showed a small amount of autolysis and yielded a negative silver nitrate test.

No explanation is advanced in the case of the patient autopsied 13 hours after death, whose vitamin C value was 0.73 mgm. per cent and whose adrenal glands nevertheless contained central cavities, and likewise in that of the patient whose adrenal glands were intact one hour after death but whose ante-mortem blood plasma contained 0.60 mgm. per cent of vitamin C. It may be that the error of the method itself (in the proximity of plus or minus 5 per cent) will not allow too rigid a line of demarcation. Possibly other and more subtle factors may have been operating to produce the exceptions noted.

The important point to be observed in the chart, however, is the remarkable division of the series into clear-cut groups having no central cavities and having central cavities, based upon the ante-mortem vitamin C content of the body. According to

E. M. Jellinek, "The probability that such a division, as shown in Figure 2, should arise by chance is less than one in a hundred* and we may say that the association between central cavitation of the adrenal glands and ascorbic acid is established beyond reasonable doubt."

SUMMARY

In a study of all autopsies (122) performed in one calendar year, we were unable to predict the presence or absence of central autolysis in the adrenal glands at autopsy, either by the state of preservation of the remainder of the cadaver, or by the length of time which had elapsed from death to examination. Neither could we find any relation between the incidence of central cavitation of the glands and the patient's age, sex, cause of death, psychiatric diagnosis, length of hospital stay, basal systolic blood pressure, basal pulse pressure, terminal febrile state, nutritional index, or total non-protein nitrogen, sugar, and chlorides of the fasting blood, or the hematocrit, hemoglobin, erythrocyte and leucocyte counts, and neutrophile percentage of the same.

In a study of 42 consecutive cases, however, it was found that the incidence of central cavitation of the adrenal glands at post mortem is directly related to the reduced ascorbic acid level of the blood plasma just prior to death. Terminal body heat and inadequate refrigeration of the body after death may influence the production of autolysis, possibly by causing more rapid auto-oxidation of the ascorbic acid.

CONCLUSIONS

A definite relationship seems to exist between the vitamin C level of the blood plasma before death and the incidence of central autolysis of the adrenal glands at autopsy. If the reduced ascorbic acid value is above 0.70 mgm. per cent no central cavities will be found in the adrenal glands, while on the other hand, if the value is below 0.70 mgm. per cent, central cavities

* Determined by the X^2 method. X^2 in this case is 46.8 (E. M. J.).

will be found. This relationship appears to be sustained regardless of the existence or absence of post mortem degeneration in the remainder of the cadaver. Such factors as terminal body heat and inadequate refrigeration may modify the relation mentioned above, presumably by hastening the destruction of ascorbic acid after death.

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THE COURSE OF BLOOD AND SPINAL FLUID GLUCOSE IN MAN (SCHIZOPHRENIC PATIENTS) AFTER SHOCK DOSES OF INSULIN*

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Most of our knowledge of the effect of shock doses of insulin on blood sugar has come from serial observations on animals and individual observations on humans (chiefly diabetic patients suffering from overdoses of insulin). Few serial observations have been made on humans, and these, like most of the individual observations, suffer from the use of methods which are sensitive to large amounts of non-glucose reducing substances. Similarly, it is to animal experiments that we owe our knowledge of the effect of shock doses of insulin on the spinal fluid glucose. Although the presence of insulin hypoglycorachia has been suspected in diabetics suffering from overdoses of insulin[1,2] and individuals suffering from hyperinsulinism, to our knowledge, no serial spinal fluid sugar determinations and only a few[3,4] individual determinations have been reported.

Since March 1936, over seventy schizophrenics have been treated in this hospital with insulin shock, and in view of the paucity of such observations the opportunity has been taken to investigate the effect of shock doses of insulin on spinal fluid sugar, as well as blood sugar, with a method which is refractory to practically all the non-glucose reducing substances of blood and spinal fluid.

EXPERIMENTAL

Simultaneous changes in the blood and spinal fluid sugar were followed in the insulin-treated patients for a period of three to four hours. The experiments were generally started about 7:00 o'clock in the morning, when a preliminary sample of blood and spinal fluid were obtained and the patient given a

* Received for publication, August 12th, 1937.

shock dose of insuln (50 to 220 units, depending upon the patient). The subsequent samples of blood and spinal fluid were taken at ½ hour intervals. In a few cases the experiments were continued to include a period or two after sugar was administered to terminate the hypoglycemia.

Blood was obtained by venous puncture from a median cubital vein, and spinal fluid by lumbar puncture. In all cases sodium fluoride was used both as preservative and anticoagulant. The sugar was determined, on Folin-Wu filtrates, by Benedict's[5] method, which according to Peters and Van Slyke[6] is refractory to all but 4–8 mgm. per cent of the non-glucose reducing substances in the blood. (Normal fasting blood sugar values by this method range from 60–90 mgm. per cent.) Because of the low sugar values obtained in many cases, half and quarter strength standards were set up, in addition to full strength standards, and the one comparing best with the sample used.

RESULTS AND DISCUSSION

Figure 1 and table 1 show the results of 28 experiments on 25 patients in which both blood and spinal fluid glucose were followed, and 3 experiments on as many patients in which only blood glucose was followed. Although the insulin dosage varied greatly, the blood sugar curves tend to be very similar. In general, the blood sugar, starting from a higher level than the spinal fluid sugar,* drops rapidly for 1 to 1½ hours, often falling below 20 mgm. per cent (expts. 17, 20, and 29, fig. 1 and expts. 3, 4, 6, 7, 23, 24, 30 and 31, table 1), and then drops slowly, eventually tending to reach a level at which it remains more or less constant. Certain small fluctuations are often found, e.g. expts. 3, 4, 8, 15, and 23, table 1, and may be the result of sympathetic stimulation caused by venous puncture. Occasionally, large fluctuations occur (expts. 11, 19, 30, and 31, table 1) and are probably attempts on the part of the body to antagonize the blood sugar decline.

Protracted experiments, 27 and 29 (fig. 1) and 4, 30 and 31 (table 1) show the extremely low level of true blood sugar which may persist for long periods, after the initial rapid drop, and

* Occasional cases are found in which the initial spinal fluid glucose is higher than the blood glucose. This reversal of levels is probably due to hyperglycemia having been present during the night[20]. Such experiments are characterized by an early and extremely rapid drop in spinal fluid glucose (Exp. 5, fig. 1 and Exp. 24, table 1).

which is endured without apparent harm to the individual. In experiment 31 we find the blood sugar lying between 14 and 7

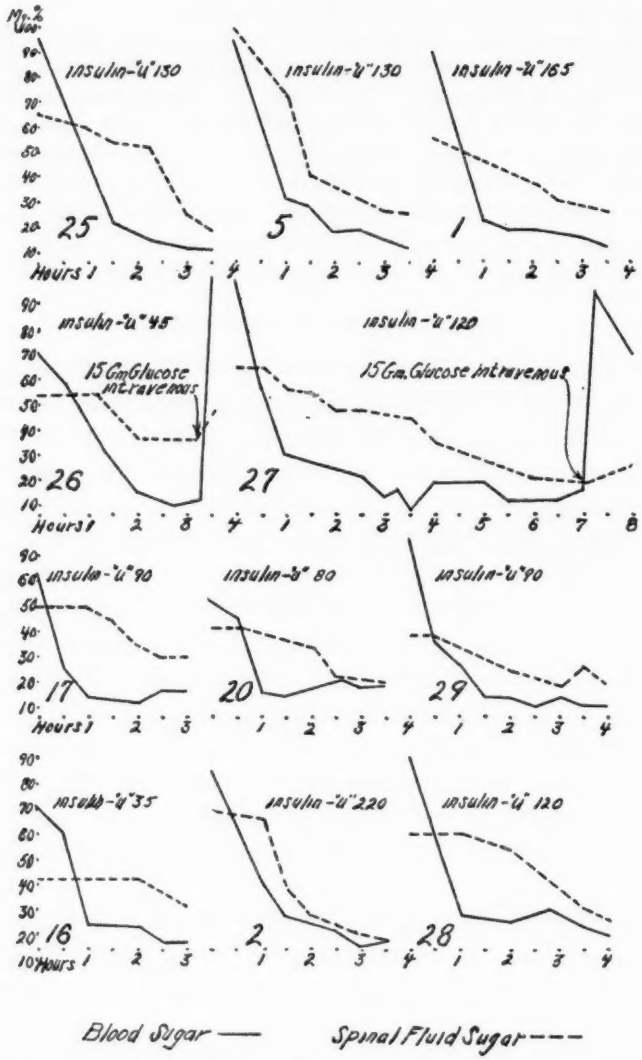

FIG. 1. BLOOD AND SPINAL FLUID GLUCOSE AFTER SHOCK DOSES OF INSULIN

mgm. per cent for 2 hours; in experiment 29 between 14 and 10 mgm. per cent for 2½ hours, and in experiment 4 between 18

TABLE 1

The course of blood and spinal fluid sugars after shock doses of insulin

EXPERIMENT NO.	INSULIN "U"		FASTING	½ HOUR	1 HOUR	1½ HOURS	2 HOURS	2½ HOURS	3 HOURS	3½ HOURS	4 HOURS
3	150	B	76	56	33	17	22	14	14	14	13
		F	50	42	42	39	50	48	44	33	24
4	120	B	125	111	53	16	13	16	18	15	11
		F	68	64	59	37	36	22	32	29	21
6	100	B	92	60	29	19	18	10	13		
		F	56	50	50	45	43	32	31		
7	50	B	80	54	26	17	17	14	13	21	
		F	39	39	39	39	39	33	30	38	
8	45	B	72	50		23	17		20	16	
		F	40	40		29	29		37	36	
9	70	B	83		35	28	24	20	20	25	
		F	78		50	43	33	35	30	26	
10	70	B	83	50	33	25	19	19	17	18	24
		F	69	69	65		47	44	37	34	32
11	80	B	94	80	42	37	13	18	40	30	
		F	80	50	47	43	27	57	42	58	
12	110	B	90	77	23	20	24	20	18		
		F	71	65	28	22	26	22	18		
13	110	B	89	77	33	25	25	24	15		
		F	48	47	36	32	24	21	17		
14	45	B	61	58	30	27	21	21	19	20	
		F	45	43	45	40	40	38	34	23	
15	110	B	82		30	35	25		33	20	20
		F	57		52	56	51		45	39	38
18	150	B	71	61	42	33	29	18	16	15	
		F	51	46		45	47		35	20	
19	90	B	71	30	15		29	23	22		
		F	53	47	30		31	27	23		
21	70	B	72		38		34	24	21	28	
		F	44		39		38	31	26	32	
22	40	B	71		49		22	26	26	26	
		F	49		44		42	40	33	33	
23	120	B	90		16	16	17	21	21	11	
		F	64		47	22	13	12	9	9	
24	100	B	42	22	22		11	12	9		
		F	63	33	30		23	17	17		
30	200	B	71			13	5	13	20	14	11
31	90	B	100			21	10	8	13	7	10
32	200	B	94			28	23	23	21	25	11

B = blood. F = spinal fluid.

and 11 mgm. per cent for 2½ hours, while in experiment 27 we find it lying below 18 mgm. per cent and as low as 8 mgm. per cent for 4½ hours. Unbelievably low as these blood sugars seem, similar values have been found in patients with hyperinsulinism by Graham and Hartmann[7] and Reinhoff and Lewis[8]. The former found blood sugars as low as 6 mgm. per cent in a year-old child subject to convulsions since 3 months of age, and the latter found blood sugars as low as 4 mgm. per cent in a patient with adenoma of the pancreas who had been having frequent attacks accompanied by total loss of consciousness, for 6 months. Even lower values are claimed to have been observed in insulinized rabbits by Dotti[9], who believes that convulsions occur only after true glucose of blood falls to zero.

Compared with the curves for blood sugar, those for spinal fluid sugar are exceedingly variable. Usually the spinal fluid sugar only starts falling 30 minutes to one hour after the blood sugar has started down; and in most cases only after the blood sugar had fallen below the spinal fluid sugar level; but there are exceptions (expts. 1, 5, and 25, fig. 1 and expts. 4, 11 and 12, table 1). It then tends to approach the blood sugar level, but the rate at which it drops varies greatly at different times in the same experiment as well as from experiment to experiment. In some cases the decline in spinal fluid sugar is gradual and regular, e.g. expts 1 and 27, (fig. 1); but in most it is exceedingly irregular, being rapid at times and exceedingly slow at other times, e.g. expts. 25, 5 and 2 (fig. 1). Cases also are encountered in which the sugar falls, rises a little, and falls again (expts. 29, fig. 1 and expts. 3, 4, 8, and 11, table 1) just as Gutowski and Wasilkowska[10] observed in dogs.

In two cases the neural fluid sugar reached the level of the blood sugar within 2 hours, after insulin, (expts. 13 and 23, table 1), and in 3 cases within 3½ hours, (expt. 12, table 1 and expts. 2 and 20 fig. 1), but in the remaining 24 cases this failed to occur although the experiments were containued for 3½ hours in 12 cases, 4 hours in 6 cases, and 7 hours in one case. We found only one case (expt. 23, table 1) in which the spinal fluid sugar was reduced more than the blood sugar. At first glance

these results appear not to be in agreement with those of Davis and Brown[1], who found that in dogs insulin often reduced the cerebro-spinal fluid sugar more than the blood sugar in less than 2 hours. However, these authors used the Folin[11] method which is sensitive to non-glucose reducing substances which amount to about 27 mgm. per cent (expressed as glucose) in blood[12] and 5 mgm. per cent in spinal fluid[13]. When corrected for non-glucose reducing substances the results of Davis and Brown are comparable to ours.

Clinically speaking, our results are described correctly. Physiologically considered, however, some modifications should be made which may be of importance in considering the mechanism of the reduction of the spinal fluid glucose. Arterial blood is generally, excepting the portal system, richer in glucose than venous blood, and insulin increases this arterial venous difference in the brachial system while it decreases it in the cerebral system[14]. This gives reason to believe that the venous blood as well as the arterial blood of meninges is richer in glucose than our analyses on brachial venous blood indicate.* With this in mind one may have to conclude that in a fair percentage of our cases the glucose of the spinal fluid fell as low as the glucose of the blood supply of the meninges, and in 5 or more cases (expts. 2 and 20, fig. 1 and expts. 12, 13 and 23, table 1) it probably fell lower than the glucose of the blood supply to the meninges. Such results suggest that utilization of glucose by the nervous system without further addition from the blood, because of its low sugar content, is responsible for the decrease in spinal fluid glucose after insulin. This assumption is supported by observations of Stewart[17] and Levinson and Cohen[18] that ventricular fluid is about 8 mgm. per cent and cisternal fluid about 5 mgm. per cent richer in dextrose than lumbar fluid. But another mechanism (diffusion of sugar from the spinal fluid to the blood) is suggested by our observations that in many cases a decrease

* We know of no work directly confirming Dameshek and Myersons observations, but the experiments of Best et al.[15] and Cori[16], showing that the chief action of insulin is to increase the oxidation and storage of glucose by the muscles, confirms them indirectly.

in spinal fluid glucose occurs only after the blood sugar has fallen lower than the spinal fluid sugar, especially when we consider that Cohen, Levinson, and McCarthy[19] and Cohen and Libman[20] have shown that glucose very readily traverses the hemato-encephalic barrier, an increase in the blood sugar causing an increase in the spinal fluid sugar.

SUMMARY

A number of schizophrenics, receiving treatment with insulin, were given shock doses of the drug and the blood and spinal fluid glucose followed for 3 to 4 hours. In general, the blood glucose was observed to drop rapidly for 1 to 1½ hours, often falling below 20 mgm. per cent, and then drop slowly to a level at which it tended to remain practically constant. The spinal fluid glucose was observed to drop very irregularly. In some cases it fell as low as the blood glucose in 3½ hours, but in most cases it did not get that low in 4 hours.

Attention is called to the extremely low blood true sugar which was endured by these patients for long periods without harm.

The probable mechanism of the reduction of the spinal fluid glucose is briefly discussed.

The authors are indebted to E. R. Squibb and Sons, for the insulin used in these experiments.

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EDITORIAL

Clinical Pathologist or Magician?

There was a time when the "laboratory" and its "staff," if not entirely overlooked, were regarded with amusement; now they enjoy the mixed blessings of such diverse emotions as contempt, respect, mistrust, confidence, depreciation, and even awe. Of course, opinion is divided but often even the same individual shuttles back and forth from one attitude to its opposite.

All in all, however, there is a sufficient number who feel that the laboratory is perhaps of some little use after all, a place in fact where magic of a sort is performed. Some may even look upon the clinical pathologist as something of a magician, though obviously not quite on the same high plane as the allergist or endocrinologist.

If we consider the fact that a few drops of blood may be made to disclose the condition of a patient's carbohydrate metabolism, a few more reveal the function of his kidneys, and that 5 cc. of urine injected into the ear vein of a rabbit may decide whether she *is*, or she *isn't*, and contemplate further the number and variety of problems (anatomical, hematological, serological, bacteriological and chemical) left at the door of the laboratory for solution, it may lead to the conclusion that perhaps this much-berated institution has some little reason for existence. Such a concession often carries the proviso that the laboratory must never err and that it must be capable of performing pretty nearly everything on a moment's notice.

Clinical pathologists, and very good ones, have confided that one of their major difficulties is the determination of lead in excreta. Somehow at the end of their efforts they are left cold, uncertain as to whether the analysis was really accurate. After several attempts they are often forced to the point of admitting their inability to undertake the procedure. Offhand it may seem

that the determination should be easy, but on closer inspection the difficulties become obvious. Usually the necessary equipment is not available even in fairly good laboratories. Besides, the laboratory may be in such close proximity to offices, wards, private rooms and clinics that the fumes and stenches incurred in such an analysis would immediately bring down upon the laboratory the wrath of all, particularly the one who requested it. In a laboratory organized and equipped to do many such analyses, the procedure soon becomes a matter of routine and presents no problem, but the condition is altogether different if a request for a lead determination comes but once in a long while. It means that someone, usually the director, must drop everything else in order to prepare or restandardize the reagents and conduct the analysis, which may consume several days. I recently inquired from the head of the chemical laboratory in a large Eastern teaching hospital whether he encountered any difficulty with the determination of lead. His reply was that the analysis was more trouble than it was worth. After a little discussion, we came to the conclusion that it might be more practical and economical if the various hospitals in this large city cooperated in maintaining a laboratory in one of the institutions where this analysis, and such others as the determination of bismuth, arsenic and mercury, might be more satisfactorily conducted.

There are many similar examples and also others of a somewhat different type. As a second illustration reference may be made to the work of several groups of investigators on the changes in blood iodine in thyroid disease. Comparison of the values obtained before and after thyroidectomy may be illuminating. It may be very interesting that in a case of hyperthyroidism the blood iodine may rise, let us say from 5γ per cent before the operation to over 100γ per cent after the operation. It must be conceded that in presenting such a case before a group of students or fellow-clinicians, data of this sort may be legitimately used in illustrating certain aspects of the pathological physiology of the gland. But is this a diagnostic procedure that can be undertaken under ordinary circumstances by the private, or

even the hospital laboratory? It is not. Irrespective of how well trained a man may be as a chemist, he must devote many days to the preparation of the necessary reagents, and many more days to acquiring the requisite technical precision. Moreover, the reagents once prepared must be scrupulously watched, protected from the slightest contamination and frequently restandardized, or renewed. It is one thing for a group of three or four investigators restricting themselves to the problem of blood iodine, equipping a laboratory specifically for this purpose, removing it sufficiently from wards and dispensaries where there is always iodine in the air, and performing their work day after day until it becomes a routine. It is a totally different thing to find, some fine morning, a 5 cc. specimen of blood with the request "Please determine the iodine content."

Some time ago a friend of mine came with the proposal that in a certain proportion of his patients it would be very desirable to analyze the urine, and perhaps even the blood, for estrogenic and gonadotropic hormones. A good idea and perhaps even clinically relevant, but imagine setting up a dozen benzene extractors daily, separating the hormones from everything else, injecting the products into series of mice and rats, taking time out for vaginal examination at 96, 104 and 128 hours, and later autopsying and exploring these animals! And where are these mice and rats to come from, who will feed them, and where and how will they be kept? That such studies, conducted under proper auspices, may be of the utmost value cannot be disputed, and it may even be granted that the technic now available may be applied in special instances to clinical diagnosis, but is it a procedure for the general laboratory of a hospital?

From the foregoing it is not to be concluded that I advocate the avoidance of newly acquired technical procedures. On the contrary progress must continue and it is often for the laboratory to set the pace. But it must be stressed that if there is to be expansion, it must be along sound and practical lines. A laboratory doing 5000 examinations a month can usually take on an additional 200 without much inconvenience. However, if the 200 additional requests for laboratory work involve unusual and

difficult manipulations, the situation becomes different. It may represent an additional load, not of 4 per cent, but of 100 per cent, or even more, and call for unavailable reagents, equipment, room, and especially skilled workers.

It is perhaps too much to hope that a laboratory will at all times run so smoothly as to meet the constant and continued approbation of all those whom it serves, but an approach to this unattainable goal may be attempted. At any rate some definition should be given to the functions and scope of the general laboratory. The work a laboratory can undertake depends on various factors. Of prime importance are the training, ability, resourcefulness, energy and interests of the director. Next is the financial support which determines the physical equipment and the quality of the technical assistance. The first aim should be organization, not for unusual procedures, but for routine work. The smooth running of a laboratory depends on how well the Wassermanns are done, how accurately, within reason, the blood counts are, whether there is enough system and sufficient help to allow the technician the necessary time for the careful examination of a blood smear, or of smears from the throat, eye, or urethra. These are among the more relevant diagnostic procedures and deserve first consideration. They are the basis of the laboratory's activities and those entrusted with these essentials should be left relatively undisturbed. The equanimity of the Wassermann technician, for example, should not be upset by being torn away from her work to do other chores thrust into the laboratory. What a laboratory is able to undertake beyond these essentials is another matter. *It should be realized that no technical procedure is simple, unless it has been a routine procedure.* Try to do even a blood count, urine analysis, or Gram stain once in a very long while and you will find it difficult and the chance for error large. The same must therefore be true, in an even greater measure, in the case of much more involved technique.

If a member of a hospital staff, or a clinician in private practice, wishes to test new laboratory methods, or to follow a legitimate problem, there should be some provision for such pursuits, how-

ever fantastic they may seem. But this should not be at the expense of the general laboratory, or of its routine work. Special projects need to be adequately subsidized, so that the technical details may be turned over to competent assistants. Many laboratory directors would be willing to act in an advisory capacity without arrogating for themselves any part of the credit that might accrue from such work.

There remains to be considered the relation of such projects to a research laboratory that may already be part of the organization. This applies usually to hospitals where provision for research is made either by the hospital itself, or where support is obtained from special endowments, or outside sources. Much depends on circumstances. As is often the case, the research laboratory may be engaged on a very definite program. With limited resources it cannot take on every problem that may be presented. Often these are of no interest to the research staff, or are foreign to the purpose for which the research appropriations may have been made. Where such situations exist, the organization of the laboratories may assume a tri-partite plan, approximately as follows:

1. The general laboratory, maintained by the hospital and engaged in routine diagnostic work.
2. The research laboratory, supported from special funds, endowments, grants in aid of research, etc. Ideally this laboratory should provide the director and his assistants an opportunity for consistent scientific investigative work.
3. A subsidiary laboratory, or group of laboratories, maintained by grants from well-disposed individuals, members of the clinical staff, foundations, etc., where problems of general or special interest to members of the clinical staff may be pursued and where certain laboratory procedures may be carried out, which are outside the scope of either the general or the research laboratory.

M. Bodansky.

NEWS AND NOTICES

The officers of the American Public Health Association announce that the 67th Annual Meeting will be held in Kansas City, Mo., October 25–28, 1938.

Dr. Edwin Henry Schorer, Director of the Kansas City Health Department, has been appointed Chairman of the Local Committee. He will be assisted by a large group of city and state officials and community leaders.

A long list of affiliated organizations meet habitually with the American Public Health Association. They include:

The American Association of School Physicians
The Association of Women in Public Health
The Conference of State Laboratory Directors
The Conference of State Sanitary Engineers
The American Association of State Registration Executives
Delta Omega
The International Society of Medical Health Officers

The attendance at the 67th Annual Meeting will exceed 3000 professional public health workers from every State in the Union, Canada, Cuba and Mexico.

The Syphilis Campaign

In the January issue information was presented concerning the formation and activities of the New Jersey Society of Clinical Pathologists with reference to the syphilis campaign.

We now summarize the activities of the Indiana Association of Clinical Pathologists, and will, in future issues, present such information concerning other groups as will, we trust, be forwarded to the Editor. What is being done in *your* State?

The Indiana Association of Clinical Pathologists

Desirous of coöperating in the syphilis campaign, the pathologists of Indiana sought and, after some delay, obtained a conference with the State Department of Health in which the following propositions were presented: (*a*) That, in order to eliminate incompetent laboratories, the State undertake the standardization of laboratories operating in the State; (*b*) that, in order to retain laboratory work in the community in which it originated, the State laboratory restrict its activities to laboratory procedures concerned solely with epidemic diseases; (*c*) that laboratory service for the indigent be financed through local, State and Federal aids.

These propositions being refused, the Association then met with the State Medical Association Syphilis Committee which later recommended to the State

Medical Association modification of State laws so that laboratory work for indigents could be financed by the State through local laboratories receiving State approval.

The proposal of pathologists that a "screen test" for syphilis be made a part of every routine examination was also accepted and recommended to the State Medical Society. The exact type of test was left to the judgment of the pathologist in question, the majority performing the Kline exclusion test. In order that the test might be available to all, a uniform price of one dollar was fixed for the "screen (exclusion) test." As false positive reactions may be obtained with such supersensitive tests, the necessity for checking positive reactions by other reliable diagnostic tests was emphasized.

At the present time, the one dollar fee is collected from the patient who also pays the balance required when an additional diagnostic test is necessary. It is hoped, however, that eventually such tests may be financed by local, state, or Federal subsidy.

Subsequent developments in this State will be summarized in these columns as they eventuate and news of them reaches the Editor.

In view of the importance of determining the most potent and most specific substance for use as an antigen in all tests for syphilis, the Serology Committee has centered its major endeavors on this problem.

Mr. La Motte, of the La Motte Chemical Products Co., has established a fellowship at Western Reserve University in the Department of Dr. H. P. Lankelma, Associate Professor of Chemistry, and John W. Wellman, Ph.D. has been selected as Fellow to engage in this research. His objectives will be: (1) The isolation from tissue extracts of the most potent and most specific substance for use as an antigen in all tests for syphilis; (2) the chemical analysis of this substance; and (3) its synthesis.

The progress of this investigation will be followed with great interest.

Hematological Loan Sets

The Hematological Research Division announces that loan sets covering the more common blood dyscrasias are now available under the following regulations:

1. A deposit of $5.00 is required to cover the cost of the slides, three dollars of which will be returned when the loan set has been returned in good condition.
2. The sets may be kept for a maximum of sixty days after which a rental charge of one dollar for each thirty days or fraction thereof will be made.

Requests for these loan sets should be addressed to:

F. J. Heck, M.D., Hematological Research Division, A.S.C.P., Mayo Clinic, Rochester, Minnesota.

Announcement has been made of the marriage of Miss Marian Archbold to Dr. Asher Yaguda at Palm Beach, Florida in January.

Technicians Institute

Believing that technicians require and will appreciate an opportunity for frequent post graduate study with special reference to the technic and practical applications of newer laboratory methods, Temple University School of Medicine is offering a Technicians Institute on April 11, 12 and 13th under the direction of Dr. John A. Kolmer and with the assistance of the Pennsylvania Society of Medical Technologists.

The first two days are devoted to lectures with an evening dinner session and the third day to laboratory demonstrations and exhibits. The course is designed to be purely practical with special reference to technic. Technicians are permitted to submit questions in writing which will be answered by the Faculty of the Institute at the dinner evening session. Registered as well as nonregistered and student technicians of Pennsylvania, New Jersey and Delaware are eligible, the fee being $2.00 ($1.00 for student technicians) and $1.00 for the dinner evening session.

The following program is being offered:

Monday, April 11th

Morning Session

10:00 Introductory.....William N. Parkinson, M.D.
Vice President and Dean of School of Medicine, Temple University

10:15 Important Technical Procedures in Blood Chemistry
Robt. H. Hamilton, M.D.
Asst. Professor of Physiological Chemistry, Temple University

11:00 Technic and Practical Value of Hormone Tests for Pregnancy
John Lansbury, M.D.
Assoc. Professor of Medicine, Temple University

11:30 Important Technical Procedures in Urinalysis..Robt. A. Kilduffe, M.D.
Director of Laboratories, Atlantic City Hospital

12:00 Technic and Problems in the Diagnosis of Allergy.....Louis Tuft, M.D.
Assoc. in Immunology, Temple Univ.; Director of Penna State Labs.

Afternoon Session

2:00 The Technic and Diagnosis of Mycological Infections
Edwin S. Gault, M.D.
Assoc. Professor of Pathology and Bacteriology, Temple University

3:00 The Newer Immunology of the Streptococci.....Stuart Mudd, M.D.
Professor of Bacteriology, University of Pennsylvania

3:30 Methods for the Preservation of Bacteria and Filtration Technic
Harry E. Morton, Sc.D.
Asst. Professor of Bacteriology, University of Pennsylvania

4:00 Methods of Anerobic Cultivation of Bacteria..Earl H. Spaulding, Ph.D.
Associate in Bacteriology, Temple University

4:30 Principles of Sterilization Applied to the Clinical Laboratory
Carl Bucher, M.D.
Asst. Director of Clinical Laboratory, Jefferson Hospital

5:00 The Technic of Venipuncture.....Samuel Sappington, M.D.
Professor of Pathology, Hahnemann Medical College

TUESDAY, APRIL 12TH

Morning Session

9:00 Hematological Standards.....Fred. Boerner, V.M.D.
Asst. Professor of Bacteriology, University of Penna.

9:45 Classification and Qualitative Changes in Leukocytes
A. J. Cresskoff, M.D.
Research Assistant in Hematology, Univ. of Penna. Hospital

10:30 Laboratory Diagnosis of the Blood Dyscrasias
Frank W. Konzelmann, M.D.
Professor of Clinical Pathology, Temple University

11:30 The Serology of Syphilis.....John A. Kolmer, M.D.
Professor of Medicine, Temple University

12:00 Technical Procedures and Diagnostic Value of Agglutination Tests
Earl H. Spaulding, Ph.D.
Associate in Bacteriology, Temple University

Afternoon Session

2:00 Methods for the Collection and Examination of Bile
B. B. Vincent Lyon, M.D.
Associate Professor of Medicine, Jefferson Medical College

3:00 An Evaluation of Methods for Gastric Analysis..William A. Swalm, M.D.
Associate Professor of Medicine, Temple University

4:00 Various Methods for the Diagnosis of Parasitic Diseases
Edwin S. Gault, M.D.
Assoc. Professor of Pathology and Bacteriology, Temple University

5:00 Recent Advances in Histo-Pathological Methods
Lawrence W. Smith, M.D.
Professor of Pathology, Temple University

Evening Dinner Session

7:30 (sharp): Dinner.
8:30 Address: The Profession of Medical Technology
Russell L. Richardson, M.D.
Asst. Professor of Medicine, Univ. of Penna.

9:00 Address: The Rôle of the Technician in the Campaign Against Syphilis
Robt. A. Vonderlehr, M.D.
Assistant Surgeon-General, U. S. Public Health Service

9:30 Address: The Value of the Institute to Technicians..Cora L. Miller, R.T.
President, Penna. Society of Medical Technologists

10:00 Answering of Questions Submitted by Technicians
Faculty of the Institute

Wednesday, April 13th

(*A*) *Demonstrations and Exhibits at Temple University School of Medicine*

(9 A.M. to 1 P.M.)

Room 504: Technic of Kolmer, Kahn and Kline Tests.....Elsa Lynch
Room 505: Technic of Anerobic Cultivation with Demonstration of Weiss-Spaulding Apparatus.....Earl H. Spaulding, Ph.D.
Room 505: Bacteriophage Technic.....Dorothy Sage, B.Sc.
Room 510: Demonstration of Animal Parasites.....Edwin S. Gault, M.D.
Room 502: Technic of Dark Field Examination for *Treponema pallidum*
Anna M. Rule
Room 502: Technic of Neufeld Method for the Typing of Pneumococci
William Good
Room 413: Technic of Allergic Skin Tests.....Louis Tuft, M.D.
Room 509: Technic of Frozen Sections.....Lawrence W. Smith, M.D.
Room 512: Pathological Museum and Methods for Mounting Specimens
Frank W. Konzelmann, M.D.

(*B*) *Demonstrations and Exhibits in the Laboratories of the Graduate Hospital*

(19th and Lombard Streets)

10 A.M. The Eagle Flocculation Test.....Marguerite Lukens, B.A.
10 A.M. Hematological Preparations Showing Various Changes in Disease
Fred. Boerner, V.M.D.
11 A.M. Technic of Various Sedimentation Tests
Chas. Jones, M.D. and Howard P. Rome, M.D.

11 A.M. Blood Bank: A Method for the Collection and Storage of Blood for Transfusions.....Paul Patton, M.D.

12 M. The Boerner-Mudd Phagocytosis Test with Special Reference to the Diagnosis of Brucellosis.....Fred. Boerner, V.M.D.

1 P.M. Preparation of Sterile Saline Solution for Clinical Use
Alex. G. Keller, Ph.G.

(*C*) *Bacteriological Department, Medical School, University of Pennsylvania*

3 P.M.: The Lyophile and Cryochem Methods for the Preservation of Complement, Sera and Cultures.....Earl Flosdorf, Ph.D.

BOOK REVIEWS

The Postmortem Examination. By SIDNEY FARBER, M.D., Associate in Pathology, Harvard Medical School. Cloth, 201 pp., 32 figures, $3.50. Charles C. Thomas, Springfield, Ill.

The purpose of this book is to present in order and in detail the methods employed in the conduct of an autopsy. The procedures described are based largely upon those employed in the Boston Children's Hospital, The Peter Bent Brigham Hospital, and the Boston Lying-In Hospital.

This manual is well and clearly written and equally well illustrated. It should prove of great value to those desirous of acquiring an efficient autopsy technic and will not be without interest or value to the skilled pathologist.

Esperimenti di Vaccinazione Antitubercolare. By PROF. ALBERTO ASCOLI, Paper, 300 pp., Milan, Italy.

This volume reports the results of experiments in vaccination against tuberculosis conducted upon calves during the last ten years at the Instituto Vaccinogeno Antitubercolare under the direction of Professor Ascoli.

The report proper is written in Italian but the principles and conclusions are summarized at the end of the book in English, French, and German.

From their experiments Professor Ascoli and his colleagues are convinced of the necessity of using attenuated live tubercle bacilli rather than heat-killed bacilli as a vaccine.

While recognizing the precautions necessary in the use of this method they are convinced of its superiority and emphasize the necessity for extensive experimental comparison of vaccination with both living (attenuated) and killed tubercle bacilli.

Maternal Deaths—The Ways To Prevention. By IAGO GALDSTON, M.D. Secretary, Medical Information Bureau, New York Academy of Medicine. Cloth, 115 pp., 75 cents. The Commonwealth Fund, New York.

This volume is intended particularly for the information of those not doctors of medicine but contains much of interest to the physician as well.

Based upon the various surveys made of this problem, the book contains all the essential facts, only technicalities being omitted.

An appendix contains the details of the community organization in Cleveland, describing both the obstetrical service in hospitals and the methods used for antepartum group instruction to mothers.

This is a well planned, sane presentation of great interest and practical value to all who are interested in this question.

Genital Abnormalities, Hermaphroditism, and Related Adrenal Disease. By HUGH HAMPTON YOUNG of The Brady Urological Institute, Baltimore. Cloth, 680 pp., 534 illustrations, $10.00. The Williams and Wilkins Company, Baltimore.

This is a volume sui generis being probably the most comprehensive treatise yet published on these subjects.

Dr. Young requires no introduction. That he has had a unique experience in the field of which this volume treats, the fifty-five cases reported in detail are evidence per se. That his experience has been utilized to the fullest extent the volume itself bears ample evidence.

To the anatomist, physiologist, pathologist and physician this book will be of great interest as a reference source; the surgeon will use it as a working text because of the surgical procedures Dr. Young has utilized in the cases reported, not only clearly described but excellently illustrated.

Excellently printed and profusely and beautifully illustrated with numerous original drawings by William P. Didusch, this book for years to come will be the outstanding reference text in this field. It can be recommended as an epochal text concerning a subject of great interest and equal importance.

The Management of The Pneumonias. By JESSE G. M. BULLOWA, M.D., Clinical Professor of Medicine, New York University of Medicine, etc. Cloth, 508 pp., 142 illustrations, $8.50. The Oxford University Press, New York.

For the past ten years Professor Bullowa, as Director of The Littauer Pneumonia Research Laboratory of the Harlem Hospital, has carried on intensive research in the various phases of pneumonia. In this volume are reported and summarized the results of these studies to date.

The book is divided into four main sections: I. Classification, Course, and Management; II. Treatment; III. Specific Pneumonias; and, IV. Prognosis.

None will dispute the importance of pneumonia as a disease. Few will dispute the importance and clinical value of this book as a comprehensive, well documented and well digested survey of extensive practical experience.

Particular emphasis has been placed on newer views and therapeutic procedures subjected to critical trial and this feature should make the book of particular value to the clinician.

This book deserves a place in every medical library.

The Cerebrospinal Fluid. By H. HOUSTON MERRITT, M.D., Assistant Professor of Neurology, Harvard Medical School, Director of the Cerebrospinal Fluid Laboratory. Boston City Hospital; and FRANK FREEMONT-SMITH, M.D., Harvard Medical School; formerly Director of the Cerebrospinal Fluid Laboratory, Boston City Hospital, with a *Foreword* by JAMES B. AYER, M.D., Professor of Neurology, Harvard Medical School Hospital. Cloth, 333 pp., 14 figures, $5.00. W. B. Saunders Co., Philadelphia, Pa.

Without doubt, this is the last word to date in books concerned with the spinal fluid. As Dr. Ayer says in his Foreword: "It is the purpose of this book to present facts. . . . It represents an honest effort to correlate recognized and well tried tests, performed under standard and personally observed conditions . . . The present book attempts to minimize such statements as are often seen in older books: 'the cells *may be* normal or increased, the pressure *may be* high or low'. The longer outlook and the method adopted here attempt to explain *why* and *at what stage* of the disease the cells are normal and *under what conditions* they are increased."

The many publications of the authors in this field, as well as their extensive experience in the Cerebrospinal Fluid Laboratory of the Boston City Hospital, establish their competence as authorities, and the book is based upon their experience in the examination of 22,000 fluids representing a varied galaxy of diseases.

This volume, as stated in its introduction, has three main aspects: the accumulation and critical analysis of data designed to reveal as much as possible of the normal physiology of the spinal fluid; the accumulation and analysis of similar data in disease as leading to an understanding of the pathological physiology of changes in the spinal fluid; the establishment of those tests which have the greatest clinical significance in the particular problem at hand.

The chapter headings follow: Anatomy and Physiology; Chemistry and Pathologic Physiology; Technic of Lumbar and Cisternal Puncture and Routine Examination of Fluid; Cerebrospinal Fluid Syndromes; Therapeutic Use of Lumbar Puncture; Roentgenography of The Ventriculosubarachnoid space; Methods.

The chapter on Cerebrospinal Fluid Syndromes alone is more than worth the price of the book, particularly for the emphasis placed on the progression of changes in the cerebrospinal fluid in relation to the various stages of the disease process, as indicating that a given change in the fluid has a different clinical significance in the early stages of the disease than it does in the terminal stages.

A comprehensive chart of "The Cerebrospinal Fluid In Differential Diagnosis" is attached to the back cover. There is a full authors index and an excellent general index.

There is no physician, no matter what his particular interest or specialized field, to whom this book will not prove of interest and value.

Some Fundamental Aspects of the Cancer Problem. Edited by HENRY BALDWIN WARD. Cloth, 248 pp.; 66 figures, $2.50. The Science Press, New York.

In this volume are gathered the papers presented at a symposium sponsored by the Section on Medical Sciences of the American Association for The Advancement of Science.

These fall into five main groups: Heredity and Constitutional Factors; Induction, Stimulation and Inhibition of Tumorous Growths; Metabolism of Cancerous Tissue; Radiation; and General Discussion of The Cancer Problem.

The contributors are all investigators of outstanding repute and the volume presents a most comprehensive survey of the present status of cancer research. As such, it is a book which no one interested in the cancer problem can well afford to be without.

Surgical Diseases and Injuries of the Genito-Urinary Organs. Sir John Thompson-Walker, Emeritus Lecturer on Urology, Kings College Hospital. Ed. 2, Revised and Edited by Kenneth Walker, Lecturer on Venereal Diseases, St. Bartholomew's Hospital. Cloth, 974 pp., 58 plates (25 in color) and 283 text illustrations, $10.00, William Wood & Co., Baltimore.

The reappearance of this well known text will be welcomed, for it has long been known as a representative and authoritative exposition of its subject.

The present edition has been thoroughly and extensively revised and with the addition of four new Chapters by the editor now reflects the recent advances and, like its predecessor, will undoubtedly take its place as a standard reference text.

The Physiological Basis of Medical Practice. By Charles H. Best, M.D. Professor and Head of Department of Physiology etc., University of Toronto, and Norman B. Taylor, M.D., Professor of Physiology, University of Toronto. Cloth, 1684 pp. 399 figures, 1 colored plate, $10.00. William Wood & Co., Baltimore, Md.

Here is a book which any physician, no matter how specialized his particular interests, may read with profit and, it may be added, with absorbing interest.

The purpose of the book is to serve to link the laboratory and the clinic and to emphasize the principles underlying diseased states.

When it is recalled that, in the last analysis, the manifestations of disease are the manifestations of disturbance, alteration of loss of function the importance and practical value of a book such as this becomes at once manifest. There is an excellent index and a complete list of references by chapters is given at the end of the text proper.

This book may be highly recommended.

Cystography and Urology. By Jas. B. McAlpine, M.D., Surgeon in charge of The Genito-Urinary Department, Salford Royal Hospital, Manchester. Cloth, Ed. 2, 487 pp. 297 figures, 14 colored plates, $9.00. William Wood and Co., Baltimore, Md.

There have been many and marked advances in the fields of cystoscopy and urography since the first edition of this book was published nine years ago, all of which are reflected in the present text.

The present volume, in addition to a thorough and extensive revision of the general text, contains much new material, notably three new chapters concerned with urography (Pelvic Resorption, Excretion Urography, and Pyeloscopy), and three discussing Fistula of the Bladder, Funnel-Neck Deformity of The

Bladder, and Congenital Abnormalities of the Kidney and Ureter. Many old figures (25) have been deleted and replaced by 141 new illustrations and two added color plates. The illustrations, excellently reproduced from equally excellent originals, are an outstanding feature of the book.

This volume deserves and will doubtless receive a cordial reception.

A Textbook of Histology. By HARVEY ERNEST JORDAN, A.M., PH.D., Professor of History, University of Virginia. Cloth, Ed. 7, 737 pp., 609 figures. D. Appleton-Century Co., New York.

That this well known text has reached a seventh edition will not be surprising to those familiar with previous editions. Those to whom it comes as a new book will find it an authoritative and comprehensive text.

The present edition contains a number of new illustrations and numerous textual additions.

The Harvey Lectures, Series XXXII, 1936–37. Cloth, 245 pp., $4.00. Wm. Wood & Co., Baltimore, Md.

In this volume are included the latest series of addresses given under the auspices of the Harvey Society and the patronage of the New York Academy of Medicine.

As usual, the contributors, include men of outstanding reputation.

The subjects are varied and include, The Cerebral Cortex and Consciousness, The Passage of Fluid Thorough The Capillary Wall, Some Functions of The Hypothalamus, The Investigation of Intermediary Metabolism With The Aid of Heavy Hydrogen, The Scientific Work of the Health Organization, The Control of Excitation In The Nervous System, The Influence of The Pituitary and Adrenal Glands Upon Pancreatic Diabetes, and Transmission of Nervous Effects by Acetylcholine.

There is thus something to appeal to every reading physician, no matter how varied his interest.

The Patient and the Weather. By WILLIAM F. PETERSEN, M.D., with the assistance of MARGARET E. MILLIKEN S. M., Volume IV. *Part I. Organic Disease. Cardio-Vascular-Renal Disease*, with a *Chapter on Experimental Endocarditis* by ALEXANDER J. NEDZEL, M.D. Cloth, 663 pp. 443 figures, $10.00. Edwards Brothers Inc. Ann Arbor, Michigan.

This book is somewhat unique both in format and content. As noted by the publishers, the format—photolithograph from perfect type-script—represents an attempt to make possible the publication of scholarly and technical books through a combination of an inexpensive process and definite economies of distribution.

To those who, like the present reviewer, have not read the preceding three volumes by this author, the book will present a novel and perhaps even an unexpected presentation the basis of which, however, becomes apparent from a careful perusal of the preface.

The present volume is in the main a presentation of evidence supporting the author's thesis which has been presented and developed in detail in the preceding volumes of the series.

As gathered from the preface, supplemented by the present text, this may be summarized as follows:

The entire series is devoted to a demonstration of the validity of the ancient Hippocratic observation concerning the association of meteorological alterations and clinical events. Underlying this thesis is the further thesis that "the single cell can follow functionally only two courses: 1) It can do more or less of its generalized or specialized function; 2) That beyond functional maxima lie regions of a) fatigue and death, and that b) beyond minima of specialized function there are apparently also stages of abnormality, undue "somnolence or inhibition; the possibility of sudden reversal to death".

Organs or tissues are aggregates of specialized cells, and what may be regarded as comparable to the survival of the fittest in a community is continually going on in an organ subjected to environmental demand. Just as the individual (an organ aggregate) is constantly subjected to unusual demands and therefore subject, in final analysis, to selection, so the individual organ (a cell aggregate) is constantly subjected to the processes of selection and survival to an even greater degree because of the rapidity of individual cell erosion and regeneration.

The basic variable resides in the oxidative mechanism and in oxygen supply —both responsible to a definite degree to environmental stimuli.

This, the author believes, leads to the probability that "(a) the individual cell or the individual organ or the totality, the individual, may be altered to the point of disease when the stimuli are of proper amplitude, frequency, or periodicity. Or, (b) when the individual, probably because of preceding injury or inadequacy, is no longer able to make the necessary adjustment to the succeeding environmental alteration." These, and similar, considerations, finally lead to the possibility "that many minor stimuli (meteorological, infectious, traumatic, emotional, etc.) acting through relatively uniform mechanisms may, by repetition and summation, ultimately cause not only dysfunction but disease, not only disease but death.

This brings with it the obvious therapeutic corollary, commented upon by Hippocrates, that the "constitution" of the winter resolves the diseases of the summer and vice versa.

"In other words, if we properly buffer our subjects in the winter we will prevent or modify the pathological conditions of the period; if we acidify or correct the accumulation of undue alkalosis of the autumn or early winter we may prevent disturbances or improve the clinical status of patients suffering from the more common conditions of that period. That lessening of stimuli (climatic change or artificially controlled environment) will prove effective; will also be obvious."

This purely Hippocratic thesis—the oldest medical thesis extant—the

author believes entirely valid and it is to demonstrate its validity that this series of volumes is presented.

The contention of the author is not that the meteorological factor is the responsible cause for all diseases but that it is a conditioning factor in a constellation of events, influencing every disease as it influences every physiological process.

In this volume he takes it for granted that, through the preceding volumes, the reader is familiar with two major concepts: "(1) the antipodal differentiation of the race (i.e. the constitution); and (2) that the meteorological environment is the most potent factor to which adjustment must be made. . . . . and that this accommodation causes energy expenditure primarily induced by transient periods of anoxemia."

In this present volume are presented numerous case histories in which the clinical course of events is correlated with coincident meteorological events shown by graphs, which the author interprets as illustrating the effect of or response to "pressor instability" "polar episodes," and "tropical episodes" consequent upon the meteorological environment.

The data presented is voluminous. Whether or not the reader is convinced of the validity of the interpretation placed upon it by the author, he cannot help but read it with interest and profit.

This is a book which requires careful reading, particularly if the reader has not seen the volumes preceding it. It is, to say the least, an interesting study and a provocative presentation of its results. With the exception of the illustrations in the Chapter by Dr. Nedzel, the figures are all either clinical charts or meteorological graphs.

The Patient and the Weather. Volume IV. *Part 2. Organic Diseases.* By WILLIAM F. PETERSEN, M. D., and MARGARET E. MILLIKEN, S. M. Cloth, 729 pp., 376 figures, $11.00. Edwards Bros. Inc., Ann Arbor, Mich.

In the review of Part I of this Volume, the authors' thesis and the evidence presented to substantiate it were outlined. This is, in brief, that disease, its manifestations, and the reaction of the patient are meteorologically conditioned. This is the Hippocratic thesis laid down in "Breaths" which the author believes is upheld by the data presented in the four volumes of this series.

As in preceding volumes case records and meteorologic records and graphs are correlated. The present volume is devoted to a study of Hypo- and Hyperthyroidism, Diabetes, The Blood Dyscrasias, and Tuberculosis.

The voluminous data collected by the author are presented and analyzed in a most interesting fashion and furnish palatable food for thought.

A Monograph on Veins. By KENNETH J. FRANKLIN, D.M., M.R.C.P., Tutor and Lecturer in Physiology, Oriel College, Assistant Director of The Nuffield Institute for Medical Research, Oxford. Cloth, 410 pp.; 45 figures, $6.00. Charles C. Thomas, Springfield, Illinois.

This, the first English monograph on this subject, is a scholarly production and a highly commendable example of the printer's art, as well.

Its object, as given by the author in his preface, is "to make available to others a somewhat recondite literature, which only a specialist could hope to summarize but which has, nevertheless, very definite bearings upon physiological, pathological and clinical problems."

Let it not be assumed from this that this book is purely of abstract and academic interest. On the contrary, the physiologist, pathologist and clinician will find in its pages much of immediate and practical value in the solution of the problems they encounter as may be seen from the following list of contents. After an historical introduction, followed by a discussion of the respective parts played by the heart, arteries, capillaries and veins, and a summary of the embryology of veins (written by Keith Richardson, M.Sc.) the author discusses the anatomy of the venous system, functionally considered; the valves in veins, blood depots and the amount of circulating blood; comparative anatomy; venules, absorption and diffusion from veins; veins and the nervous system; the heart and the venous return; the effects of hydrostatic pressure; the effects of the contraction of voluntary muscle on venous return; the effect of contraction of involuntary muscle on venous return; pulsation of arteries acting on veins; functional variations in length of veins and their effects upon venous return; respiration and the venous return; venous pressure; the movements of blood in the veins; clinical considerations and notes on the application of photographic technics to research on the venous system.

It is apparent that the question has been thoroughly surveyed and the text evidences that it is competently discussed.

Both author and publisher are to be congratulated upon a production of outstanding merit.

CLINICO-PATHOLOGIC APPLICATION OF SERUM PHOSPHATASE DETERMINATIONS, WITH SPECIAL REFERENCE TO LESIONS OF THE BONES*

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Many reports have indicated the relation of increased phosphatase activity of the blood to diseases in which there is new bone formation; to conditions in which the body apparently attempts to lay down new bone; and to diseases of the liver and biliary tract. It is also well known that serum phosphatase activity is influenced by age, malnutrition, cachexia, and the nature of the diet. These findings have been adequately discussed and need not be reviewed.

It is the purpose of this paper to suggest the adoption of a simple technic for the estimation of the phosphatase activity of blood serum which will permit a uniform expression of results; to present certain modifications in chemical procedure; to report certain cases of our own in which the estimation of the serum phosphatase gave valuable diagnostic aid; and to urge a more widespread clinical use of this laboratory procedure.

The earlier studies of phosphatase in bone disease were carried out by the method of Kay.[1] With this method much valuable information was obtained, but in its use certain inaccuracies arise which have been pointed out by Bodansky.[2] The Kay method uses a 48-hour period of hydrolysis of the organic phosphate substrate. This long hydrolysis period is impractical for clinical purposes and it is wholly unnecessary because accurately determinable increases in the phosphate content of serum are obtained

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with a one-hour incubation period. And still more important, the results of a long hydrolysis period are much less accurate than those obtained by a short hydrolysis. Bodansky has shown that a variable retardation of the action of this enzyme by the products of its hydrolysis occurs which gives inaccurate values with sera high in phosphatase content. The same objections apply to the method of Lundsteen and Vermehren[3] in which a 24-hour incubation period is used.

The advantages of a short hydrolysis period are found in the methods of Bodansky[2], Roberts[4], Jenner and Kay[5], and King and Armstrong[6].

In the determination of phosphatase the buffering of the serum-substrate mixture is of importance. The optimum pH for human phosphatase activity, according to Kay[1], is 8.8 to 9.2. In the Kay method the pH of the blood serum or plasma is determined and results are calculated by interpolation to a pH of 7.6. Woodard, Twombly, and Coley[7] determine the phosphatase activity and the pH of two serum-substrate mixtures buffered to approximately pH 8.2 and 8.7 and calculate the activity at pH 8.6 by interpolation. A separate pH determination is added work and fails to obtain the desired advantages since a sample of blood serum or plasma undergoing autolysis in an incubator does not maintain a constant pH. A better solution of this difficulty is the use of a buffer which will hold the pH of the mixture undergoing hydrolysis at the optimum pH for phosphatase activity and the use of a buffer has now been adopted by most authors[2,3,4,5,6]. Bodansky[2] has raised objections to the glycine buffer used by Jenner and Kay, claiming that low results are obtained by its use.

It is regrettable that much confusion exists in the literature because of a difference in the value of the phosphatase unit. An examination of tables 1 and 2, which show the value of the phosphatase unit of different authors, will reveal the difficutly of making comparisons of published results. The lowest normal value for the adult ranges from 0.10 to 0.21 units (Kay); and the highest normal value varies from 33 to 49 units (Lundsteen and Vermehren). For children the lowest normal value varies

from 0.17 to 0.34 Kay units; while the Lundsteen and Vermehnen normal value shows a range of 156 to 241 units. Satisfactory

TABLE 1

PHOSPHATASE UNITS

AUTHOR	DEFINITION
Kay	Amount of enzyme liberating 1 mgm. P at 38°C., pH 7.6, in 48 hours
Lundsteen and Vermehren	Amount of enzyme liberating 1 mgm. P at 37°C., pH 8.87, in 24 hours
Jenner and Kay	Amount of enzyme liberating 1 mgm. P at 38°C., pH 8.8, in 3 hours
Roberts	Amount of enzyme liberating 1 mgm. P at 38°C., pH 8.9, in 2 hours
Bodansky	Amount of enzyme liberating 1 mgm. P at 37°C., pH 8.6, in 1 hour
King and Armstrong	Amount of enzyme liberating 1 mgm. Phenol at 37.5°C., pH 9.0, in ½ hour from di-sodium phenyl phosphate

TABLE 2

PHOSPHATASE NORMAL VALUES

AUTHOR	NORMAL VALUE FOR THE ADULT
Kay	0.10 to 0.21 units per 1 cc. of plasma
Lundsteen and Vermehren	33.0 to 49.0 units per 100 cc. of plasma
Jenner and Kay	3.2 to 7.9 units per 100 cc. of plasma
Roberts	2.0 to 5.5 units per 100 cc. of plasma
Bodansky and Jaffe	1.5 to 4.0 units per 100 cc. of serum
King and Armstrong	3.7 to 13.1 units per 100 cc. of serum
	NORMAL VALUE FOR CHILDREN
Kay:	
Under 1 year	0.50 to 1.14 units per 1 cc. of plasma
Children	0.17 to 0.34 units per 1 cc. of plasma
Lundsteen and Vermehren:	
Under 1 year	156.0 to 241.0 units per 100 cc. of plasma
3 to 13 years	43.0 to 147.0 units per 100 cc. of plasma
Bodansky and Jaffe:	
2 to 15 years	3.1 to 13.1 units per 100 cc. of serum

comparisons of results obtained by long hydrolysis periods cannot be made with results determined by short incubation periods

because the values are not linear with respect to time. The adoption of a unit similar to the Bodansky unit based upon a one-hour incubation period would seem to be a satisfactory solution of this situation. A one-hour incubation period gives an ample increase in organic phosphate for reliable quantitative work; it is admirably adapted to clinical work where prompt and sometimes frequent determinations are desirable; and the short incubation period gives more accurate results since it does not incur the variable retarding influence of increases in inorganic phosphate upon the hydrolysis of phosphate substrate. Results obtained by a hydrolysis period that either accidentally or intentionally exceeds one hour may be calculated to a one-hour basis by the correction data published by Bodansky[2], *provided the conditions of the procedure are similar to the Bodansky method.*

In our own work we have used the Bodansky method modified by the use of the Fiske and Subbarow[8] technic for the determination of inorganic phosphate instead of the Kuttner and Lichtenstein[9] procedure for inorganic phosphorus. Several C. P. grades of molybdate salts examined by us gave a blue color when mixed with acid and the Kuttner and Lichtenstein stannous chloride reagent. Kuttner and Lichtenstein explain the blue blank obtained with sodium molybdate as being due to "soluble tungsten salts or to a substance containing iron, tungsten and silica." Bodansky, in attempting to overcome this objection to sodium molybdate, used a very pure grade of molybdic acid dissolved with enough sodium hydroxide to make the solution alkaline to phenolphthalein. Bodansky's modification does not remove the objections to this reagent, however. We found the blank obtained with sodium molybdate increases as this reagent is allowed to stand in the bottle because sodium molybdate is alkaline and dissolves silicates from the glass bottle in which it is kept and silicomolybdic acid gives a blue color with stannous chloride. As Bodansky makes his sodium molybdate reagent "alkaline to phenolphthalein," silicates will also accumulate in his reagent upon standing, making it unsatisfactory upon aging unless kept in a non-glass container.

Woodard, Twombly and Coley[7] were unable to obtain a color-

less blank test with the Kuttner and Lichtenstein reagents and to overcome this difficulty they determined the value of the blue blanks produced with those reagents and made appropriate corrections. When these corrections were applied, it was found that the deviation from Beer's law by the colors obtained with varying concentrations of standard phosphate solution was much less than that reported by Bodansky. The use of correction data is tedious, however, and unsatisfactory in this instance unless the corrections are frequently made, since they do not remain constant with these reagents.

In view of these facts it seemed desirable to adopt the more specific Fiske and Subbarow technic for inorganic phosphate determination. Using the same molybdate solutions which gave a blue color with the stannous chloride reagent of Kuttner and Lichtenstein we obtained a colorless blank with the Fiske and Subbarow 1-amino-2-naphthol-4-sulfonic acid reagent. Ammonium molybdate, which is used by Fiske and Subbarow, is also a better reagent than sodium molybdate which is used in the Bodansky method, because ammonium molybdate solution is not alkaline and therefore does not accumulate silicates upon standing in a glass bottle. Our sodium molybdate, when freshly prepared, did not give a blue color with the Fiske and Subbarow reagent, but after standing in a glass bottle for some months it gave a blue blank test; our ammonium molybdate, on the other hand, gave a colorless blank after 1 year's standing when tested with this reagent.

We have modified the Fiske and Subbarow method by using trichloroacetic acid in the standard solutions instead of sulfuric acid. This makes conditions in the standard and the unknown more uniform as to quality of the acid and the pH of the resulting solutions, which should be approximately the same. Of course, a pure grade of trichloroacetic acid, free from phosphate or other impurities which may either accentuate or diminish the blue color produced, should be used, but ideal conditions may not always exist and it is therefore desirable in colorimetric methods to make conditions in the standard and unknown as nearly uniform as possible by using similar reagents in both.

In our cases we have taken into consideration the non-osseous factors which influence the level of serum phosphatase such as age, state of nutrition, diseases of the liver and biliary tract. No jaundice cases were studied. For normal values we have used the data of Bodansky[2]: 1.5 to 4.0 units for adults and 3.1 to 13.1 units for children, per 100 cc. of serum.

TECHNIC FOR SERUM PHOSPHATASE DETERMINATION

Reagents

1. *Trichloroacetic acid.* A pure grade of trichloroacetic acid, free from phosphate, must be used. Prepare accurately a 5 per cent and a 10 per cent solution.

2. *Buffered glycerophosphate substrate.* 2.15 grams of sodium glycerophosphate and 2.12 grams of sodium diethyl barbiturate are dissolved in 500 cc. of distilled water. Cover with a 3 cm. layer of petroleum ether and keep in a refrigerator.

3. *Molybdate reagent.* Dissolve 2.5 grams of ammonium molybdate, C.P., in 100 cc. of distilled water.

4. *Aminonaphtholsulfonic acid reagent.* Dissolve 30 grams of sodium bisulfite, C.P., and 1 gram of sodium sulfite, C.P., in 200 cc. of distilled water. Add 0.5 gram of purified 1-amino-2-naphthol-4-sulfonic acid and mix thoroughly. Filter. Place in a dark bottle. This reagent should be freshly prepared about once a month.

5. *Standard phosphate solutions.* (a) *Stock solution:* Dissolve 4.388 grams of pure dry KH_2PO_4 in 1 liter of distilled water. Add 5 cc. of chloroform to prevent mold formation. One cubic centimeter of this solution contains 1 mgm. of phosphorus. (b) *Dilute standards for blood phosphatase:* Dilute 1 cc., 2 cc., 3 cc. and 4 cc. of the stock solution to 500 cc. with the 5 per cent trichloroacetic acid prepared above. Five cubic centimeters of these diluted standards contain 0.01, 0.02, 0.03 and 0.04 mgm. of phosphorus, respectively.

Procedure

Pipette 10 cc. of the glycerophosphate substrate into a test tube and place in a beaker of water at 37°C. for a few minutes. Add 1 cc. of serum (or plasma), mix thoroughly, replace in the beaker of water and set the beaker in an incubator at 37°C. for 1 hour. After 1 hour remove from the incubator and add 9 cc. of 10 per cent trichloroacetic acid. Mix thoroughly, let stand for 2 minutes, and filter through a phosphate-free filter paper (Whatman's No. 44). This filtrate is used for determining the original phosphate of the serum plus the phosphate resulting from hydrolysis of glycerophosphate by the phosphatase. Label it phosphatase filtrate.

To 1 cc. of serum add 9 cc. of 5 per cent trichloroacetic acid, mix thoroughly, let stand for 2 minutes, and filter through a phosphate-free filter paper. This filtrate is used to determine the inorganic phosphate of the serum. Label it control filtrate.

For the determination select tubes graduated with a 10 cc. mark. Place 5 cc. of the control filtrate in a tube labeled appropriately and 5 cc. of the phosphatase filtrate in another tube.

Prepare standards by placing in tubes 5 cc. portions of the diluted standard phosphate solutions, making standards available which contain 0.01, 0.02, 0.03 and 0.04 mgm. of phosphorus per 5 cc.

To each of the tubes containing standard or filtrate add 1 cc. of molybdate reagent and 1 cc. of aminonaphtholsulfonic acid reagent. Make all tubes up to 10 cc. with distilled water and mix thoroughly. After 5 minutes read in a colorimeter, selecting for each unknown the standard which most closely matches it.

Calculation

For control filtrate:

$$\frac{S}{U} \times S_1 \times 200 = \text{mgm. per 100 cc. of serum.}$$

For phosphatase filtrate:

$$\frac{S}{U} \times S_1 \times 400 = \text{mgm. of P per 100 cc. of serum plus P liberated by the enzyme.}$$

(S = reading of standard; U = reading of unknown; S_1 = mgm. P in standard selected.) To obtain the units of phosphatase subtract the value of the control filtrate from that of the phosphatase filtrate.

CLINICAL APPLICATION

It has been shown, as summarized by Franseen and McLean[10], that phosphatase is formed by the osteoblasts, and that it is an important factor in ossification. As a result, phosphatase determinations would be expected to be of special value in the study of lesions of bone; and, in fact, intensive study of phosphatase has been made in such lesions.

Phosphatase is high in conditions of active bone formation, and in conditions of "frustrated attempts at osteogenesis." It is high in rickets, hyperparathyroidism[11] (generalized osteitis fibrosa cystica, von Recklinghausen's disease of bone), osteitis deformans (Paget's disease of bone), and in the osteoblastic type of osteogenic sarcoma.

One of our cases of *osteitis deformans* illustrates this point.

Case 1. A. D. A 58 year old white woman. Was sent into the hospital with a diagnosis of traumatic epilepsy. She walked into the hospital with assistance. Her complaint was weakness, shaking spells, dizziness, headache, and roaring in the ears and head. She sometimes falls with these spells, which

FIG. 1. (Case 1.) "Skull shows evidence of a marked thickening of the cortical plate, the skull measuring nearly one inch in thickness."

come on several times a day. About a month ago, she fell in one of these spells, and struck her head on a table. She was treated in another hospital for the resulting scalp wound, and remained in that hospital for about two weeks.

X-ray examination showed the appearance as in figures 1, 2, and 3. Serum phosphatase was 13.9 units; calcium 12.5 mgm., phosphorus 4.0 mgm.

Phosphatase is increased in osteoblastic metastasis of carcinoma to bone, as in most prostatic cancers; not increased in osteolytic metastasis of carcinoma to bone, as in most breast cancers[12].

Phosphatase is high in some *metastases of mammary cancer to bone*, as illustrated by one of our cases.

Case 2. C. D. A 64 year old white woman. She had a large stony hard cancer of the left breast, of 15 years duration. There were numerous enlarged glands in the left axilla. She began having pain in the lumbar region about six months before entering the hospital.

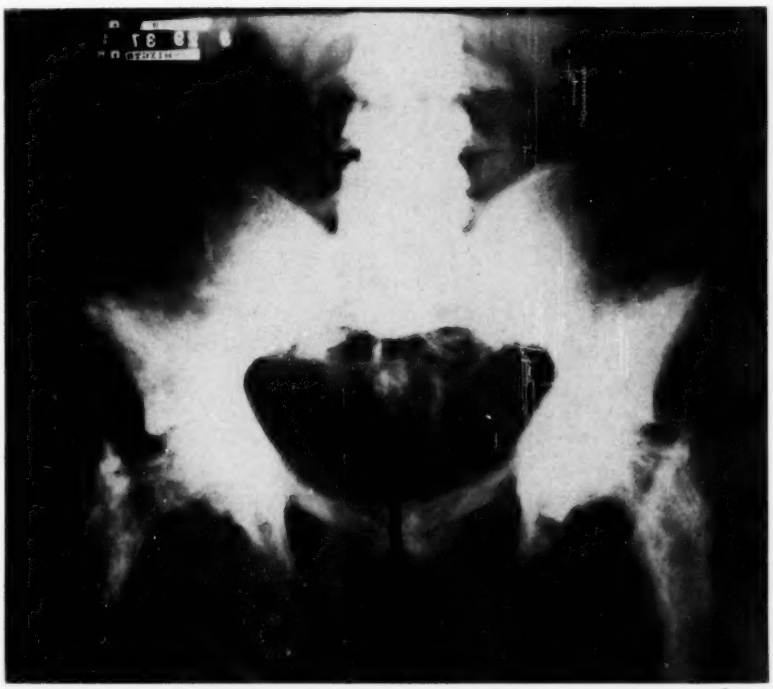

FIG. 2. (Case 1.) "The lower three lumbar vertebrae, sacrum, bones of pelvis, upper thirds of the femora, show extensive osteoblastic and osteoclastic changes, with a marked increase and prominence of the bony trabeculae."

X-ray examination showed metastases as in figures 4 and 5. Serum phosphatase 6.6 units.

There is no increase in phosphatase in *carcinoma with metastases other than to bone*, as illustrated by another of our cases.

Case 3. A. O. B. A 32 year old colored woman. She was at another hospital, with a diagnosis of carcinoma of the cervix uteri, and was treated with X-ray and radium on this malignancy. No X-ray examination of the chest

FIG. 3. (Case 1.) The upper thirds of both tibias show the changes described under figure 2; and "the tibias show marked evidence of anterior bowing, with marked thickening along the posterior and concave surfaces."

FIG. 4. (Case 2.) "Extensive metastases throughout the vertebrae."

was made in that hospital. She entered this hospital seven months later; and X-ray examinations of her chest showed the condition as in figure 6. Serum phosphatase 0.5 unit.

In this connection, the study of plasma phosphatase in carcinoma, by Bowman and Pitts[13] is of interest. In two cases the phosphatase was high (one carcinoma of the uterine cervix, one

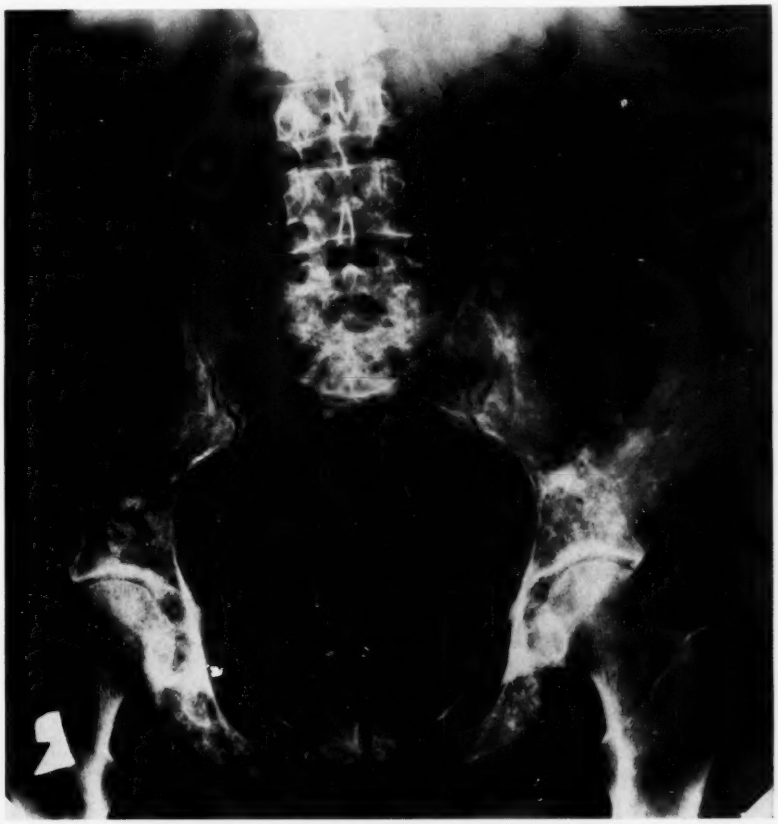

FIG. 5. (Case 2.) "Extensive metastases throughout the pelvic bones."

carcinoma of the body of uterus), and was moderately high in one case (carcinoma of the uterine cervix with possible metastasis to the liver*). It would be of interest to know whether in these cases there was metastasis to the bones.

* This case brings into consideration the question of high phosphatase readings in diseases of the liver, especially with jaundice; for discussion of which, reference is made to Cantarow's review.[14]

The fact that "variable slight elevations of plasma phosphatase occur in fracture repair,"[15] led us to consider the study of serum phosphatase in ununited fractures in elderly persons; and one of our cases illustrates this point.

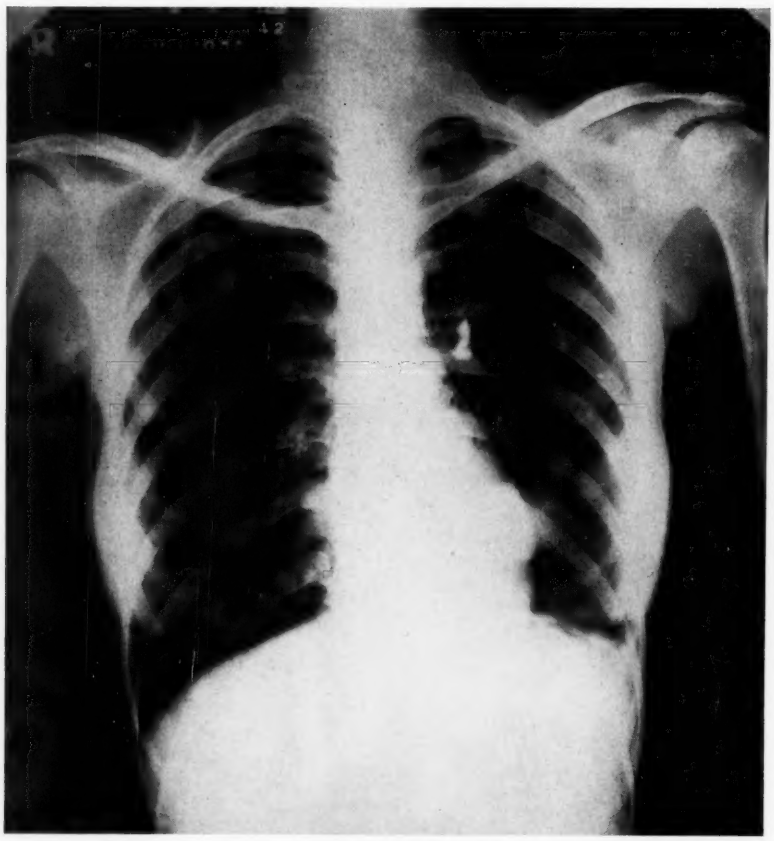

FIG. 6. (Case 3.) "A large round shadow of increased density at the left base, approximately 3 cm. in diameter, the borders of which appear round, smooth and regular. There are also two small similar shadows beneath this large shadow, just above the diaphragm, and another small shadow at the right base. Indicative of carcinomatous metastasis."

Case 4. C. E. W. A 68 year old white woman. She fell and injured her hip. X-ray examination showed an intracapsular fracture of the neck of the left femur, with absorption of the neck of the femur, as in figure 7. Serum phosphatase 1.5 units.

Wheeldon's work on stimulation of repair in fractures[16] led a member of our hospital staff to undertake similar work; and we

FIG. 7. (Case 4.) "Absorption of the neck of the femur, with no evidence of bony union."

have undertaken to follow the behavior of the serum phosphatase in his cases, one of which is cited here.

Case 5. A. P. A 50 year old white woman. Three years ago, she fell on the floor, and fractured the neck of her left femur. X-ray examination on entering this hospital, one month ago, showed the condition as in figure 8. Phosphatase 1.23 units.

She was given 4 cc. of splenic extract, by mouth, three times a day, for one month. As there was no apparent improvement, an operation was done on

FIG. 8. (Case 5.) "Intracapsular fracture of neck of left femur; no evidence of fibrous or callus union."

the hip, and the patient died of shock. Blood was taken six hours post mortem for phosphatase determination. The analysis showed 11.2 units.

The serum inorganic phosphorus was abnormally high, but the phosphatase activity was undoubtedly increased.

Phosphatase studies are of great value and interest in the study of diseases of the lymphoid and myeloid systems, with bone lesions. Phosphatase is normal or slightly elevated in multiple myeloma; so phosphatase determination is of value in differen-

tiating multiple myeloma from hyperparathyroidism[17]. Phosphatase is normal in Ewing's sarcoma; normal or slightly elevated in benign giant cell tumor.

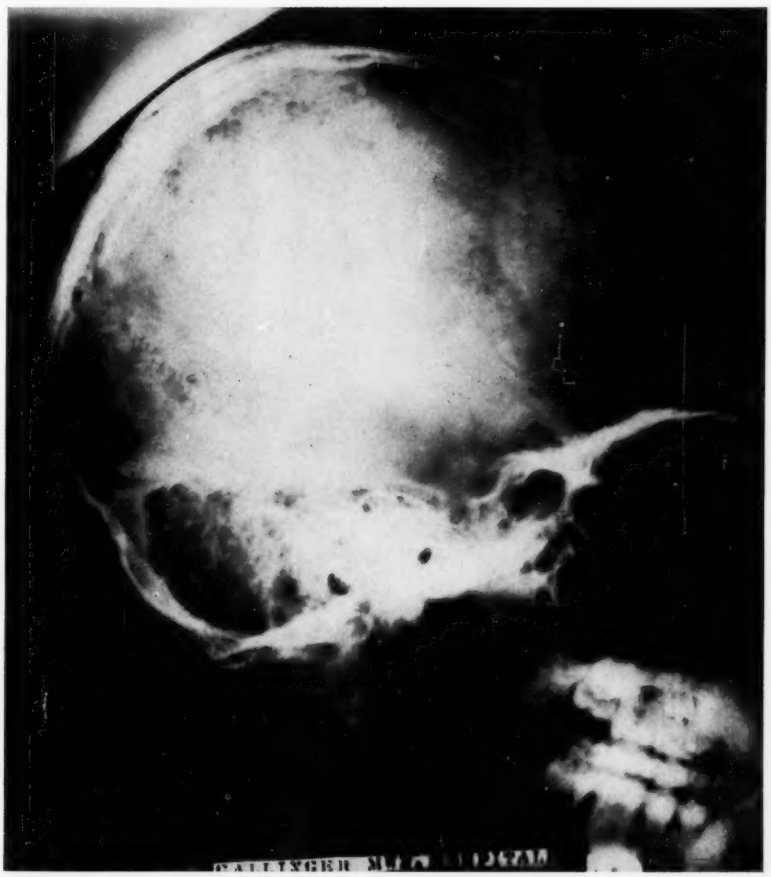

FIG. 9. (Case 6.) "The skull shows extensive osteoporosis, with softening and diffuse irregular cortical absorption. The bones present a mottled moth-eaten appearance."

One of our cases of *aleukemic leukemia, lymphoid type, with bone involvement,* is of interest here.

Case 6. J. E. A 9 year old white girl. Was in the hospital for removal of tonsils and adenoids; she was in the hospital nine days, with nothing of comment.

She reëntered the hospital nineteen days later, with an admission diagnosis of arthritis. There was anorexia, vomiting, weakness; she did not want to stand or walk; complained of pain in the chest anteriorly at the lower costal

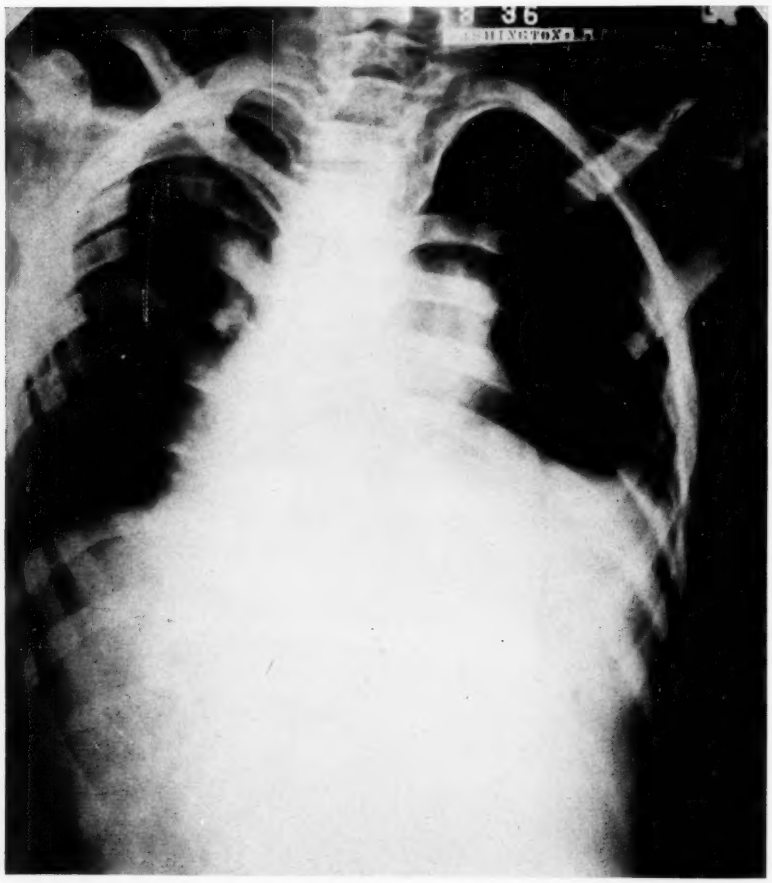

FIG. 10. (Case 6.) "The chest shows no evidence of infiltration, consolidation, or effusion. The heart is not enlarged. The diaphragms are clearly outlined."

margins; complained of pain on being moved; and cried out with pain at night. She complained of various pains.

X-ray examination showed the condition as shown in figures 9, 10, 11, and 12.

Blood examination, ten days after admission: red cells 3,160,000; white cells 7000; differential; polymorphonuclears 61, band forms 8, lymphocytes 27,

monocytes 4. Serum phosphatase 1.3 units; blood calcium 11.1 mgm., phosphorus 4.3 mgm. Urinalysis showed a trace to a fair amount of albumin; Bence-Jones protein absent in two tests.

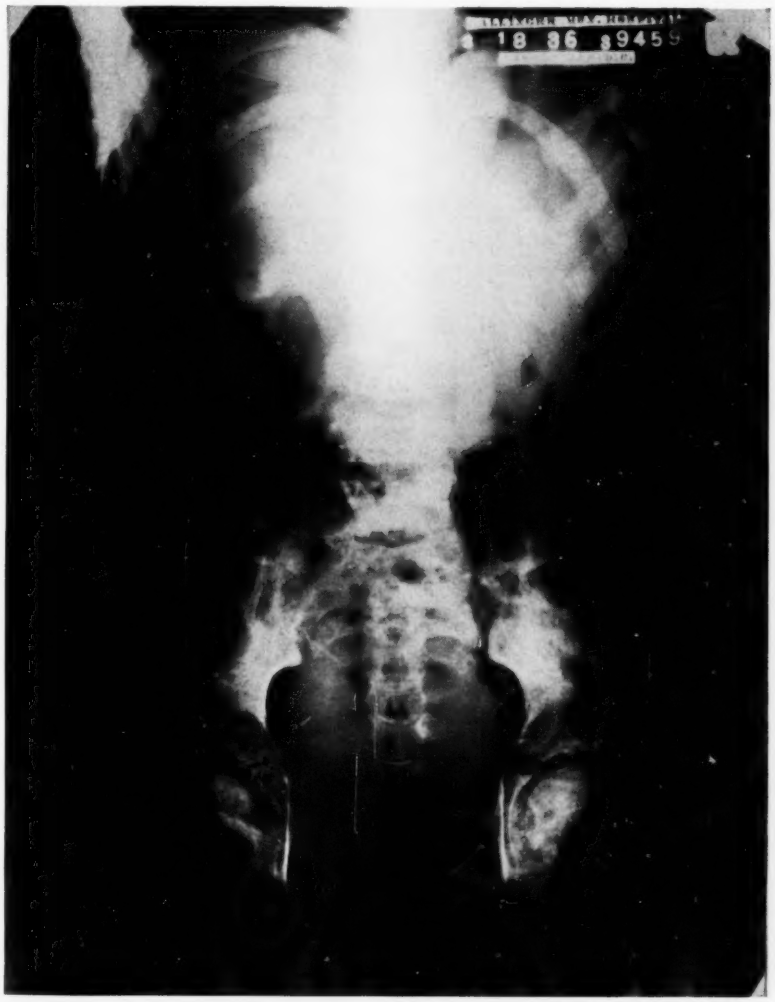

FIG. 11. (Case 6.) The pelvis, spine and ribs show the same changes as described for figure 9. "There is considerable evidence of softening and compression of the vertebrae."

Blood examination three weeks later; red cells 2,950,000; white cells 14,000; differential: polymorphonuclears 56, band forms 13, eosinophiles 3, lymphocytes 21, monocytes 7.

An axillary gland was removed and sectioned, with the picture shown in figure 13. There was considerable enlargement of the lymph nodes especially in the abdomen before death. A piece of the twelfth rib, on the right side, was removed and sectioned, with the picture as shown in figure 14.

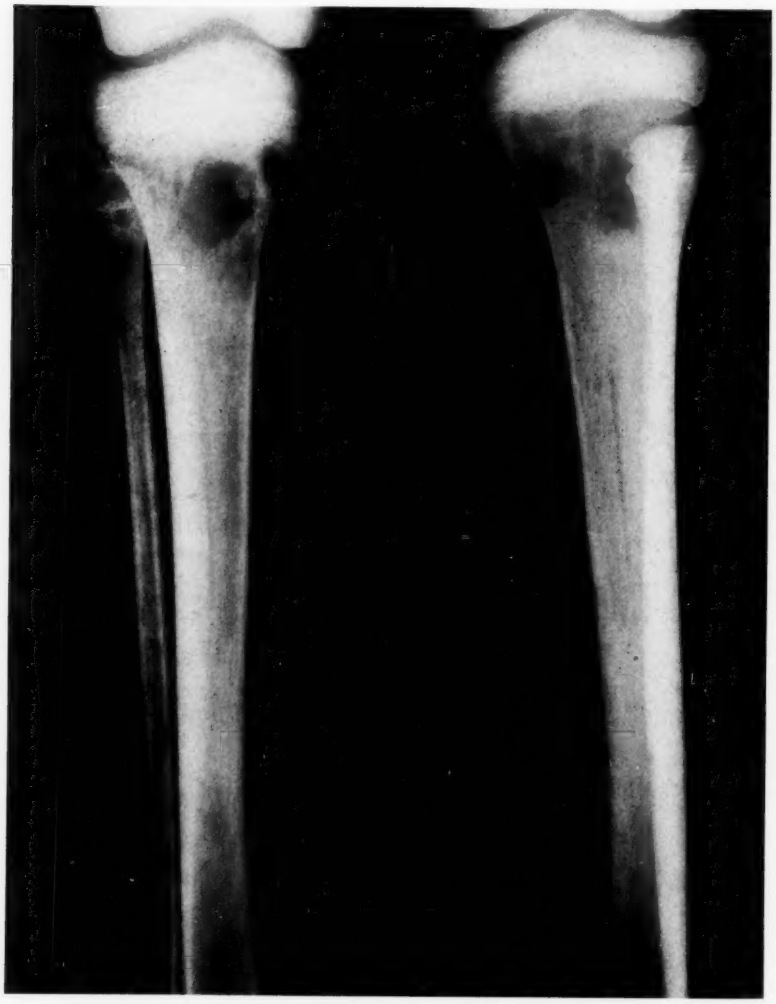

FIG. 12. (Case 6.) "The upper ends of the tibias and fibulas show large cystic areas of bone resorption. The epiphyses are not involved. The upper ends of the humeri, and the upper and lower ends of the femora, show the same changes. The hands and feet are free of this wide-spread cortical destruction."

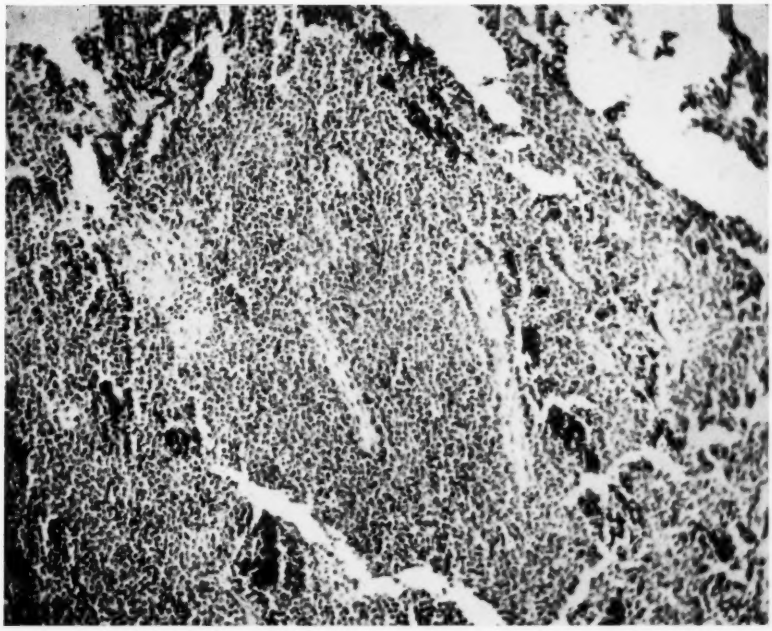

FIG. 13. (Case 6.) Biopsy. Axillary lymph node. Architecture of lymph node gone. Uniform sized cells, uniformly distributed. No hyperchromatosis; no mitoses.

FIG. 14. (Case 6.) Biopsy. Rib. Erosion of the bony trabeculae, with intense small mononuclear round cell infiltration in the marrow spaces. No osteoblasts are seen.

The patient died, seven weeks after entering the hospital the second time. Necropsy was not allowed.

Clark[18] reports a case similar to ours, with high blood calcium (19.3 mgm.) and 4.5 mgm. of phosphorus. No determination of phosphatase was made. The leucocyte count was 6,130: differential: polymorphonuclears 62, transitional 2, eosinophiles 3, leucocytes* 33. On account of the high blood calcium, and the bone involvement, hyperparathyroidism was suspected, and an operation was done; a small tumor was removed from the region of the parathyroids, but microscopically no parathyroid tissue was found in this mass. Four days after the operation, the child's leucocyte count rose to 25,800, with 69 per cent lymphocytes.†

Since there was no increase in phosphatase in our case, and phosphatase is high in hyperparathyroidism, it seems that a phosphatase determination in such cases is an aid in differentiating the condition from hyperparathyroidism.

SUMMARY

1. The phosphatase activity of blood serum is conveniently and rapidly determined. There should be a more wide-spread clinical use of this laboratory method. Confusion exists in the literature concerning phosphatase data because of the wide differences in the value of the phosphatase units of various authors. The adoption of a unit similar to the Bodansky unit, based upon a one-hour incubation period, seems desirable. Certain modifications in the Bodansky phosphatase technic are recommended.

2. Phosphatase is associated with osteoblastic activity; hence, the determination is of special value in lesions of bone. Published reports are cited, and comparable cases are cited from our own experience, indicating where phosphatase determinations are of value in differential diagnosis.

* Lymphocytes.

† Clark says that Doctor George Minot made a definite diagnosis of aleukemic leukemia on a similar case in the Children's Hospital in Boston.

3. A case of aleukemic leukemia, lymphoid type, with extensive involvement of the bones, is reported, in which serum phosphatase was in the lower part of the normal range for persons of the age of the patient. The serum phosphatase determination here aided greatly in differentiating the case from hyperparathyroidism.

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OSTEOPETROSIS (MARBLE BONE DISEASE)*

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In 1904 Albers-Schönberg,[1] a roentgenologist of Hamburg, described a disease affecting the osseous system which he named marmorknochen-krankheit or "marble bone disease." According to Wortis[2] who reviewed the subject in 1936, somewhat less than eighty additional cases have appeared in the literature under such names as Albers-Schönberg disease,[10] Osteosclerosis fragilis generalasata,[3] Osteopetrosis,[4] Congenital osteosclerosis,[5,6] "Chalky bones,"[7] and "Marble bones."[8]

The disease is characterized by an endosteal increase in the thickness and density of the skeletal system, and by profound hematopoietic disturbances. All bones are affected, but the involvement is more striking in the vertebrae, pelvic bones, base of the skull, proximal ends of the femurs, and distal ends of the tibiae. Individually, the bones appear opaque in x-ray films with partial or complete obliteration of the medullary cavities. When the disorder is fully developed the normal bone markings are obliterated and to some extent replaced by trabeculations. The opaque shadows of the long bones are characterized further by parallel transverse striations in the epiphyseal areas, which represent alternating planes of greater and lesser densities.

An array of secondary and associated manifestations of osteopetrosis follow the bone changes like shadows. Among those of a secondary nature are retarded growth, pathologic fractures, optic atrophy, hydrocephalic changes, chronic osteomyelitis and

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imperfect dentition. The gravest symptoms, however, arise from the associated hematopoietic disturbances which are accompanied by severe anemia, leukemic states, and enlargement of the liver, spleen, and lymph nodes. Formerly regarded as being of secondary or compensatory nature, it is more probable that these hematopoietic changes are a part and parcel of the disease.

ETIOLOGY

The ultimate cause of osteopetrosis is not definitely understood. Serologic evidence of syphilis has been lacking in all but a very few of the reported cases. Disturbances of calcium [9, 10] and of phosphorus[11] metabolism resulting in excessive accumulations in the bones have been held to be responsible factors. On the other hand, results of critical investigations of calcium metabolism by Laurell and Wallgren[12] were considered by them to fall within normal limits. Regardless of the evidence for or against calcium or phosphorus metabolic disorders, the question remains, as pointed out by McCune and Bradley,[13] whether these disturbances are, in fact, cause or effect. The same questionable deduction applies to theories of endocrine dysfunction,[14, 15, 16, 30, 32] and is pertinent also to investigations of vitamin deficiency.

From a review of many published case reports, one can not escape the implications of certain genetical relationships and developmental aspects of the disease. Parental consanguinity has been rather frequently reported. In our case the patient's great-grandfathers were brothers. Pirie's[7] oldest patient with marble or chalky bones was the mother of three similarly afflicted children and roentgenographic evidence was secured of the existence of the disease in a child in utero. Ghormley[6] reported the disease in a father and son and Alexander[31] a case in the mother of five presumably normal children. The disease may or may not be directly transmitted to offspring. Among the reported cases, the number of males and females is about equal, indicating thereby that the hereditary element, whatever it may be, is not sex-linked. If a disturbance in the germ plasm be assumed, this constitutes genetically a Mendelian recessive characteristic and it follows that the inbreeding of this trait makes probable a

higher degree of incidence. McCune and Bradley[13] further support the theory of familial and hereditary influence by citing the close analogy to the operation of these influences in osteopsathyrosis (osteogenesis imperfecta) in which the resulting bone changes are exactly the reverse of those in osteopetrosis.

If one postulates the initial defect in the germ plasm, the development of the disease along characteristic lines may be explained, as Klemperer has suggested in his discussion of a case report by Alter et al.[19] In substance Klemperer pointed out that, since undifferentiated mesenchyme is the common progenitor of both blood and bone, a perversion of normal development may manifest itself in an excessive growth of osteogenic tissue at the expense of the bone marrow. The relatively fibrous marrow which does develop seems to be more responsive to metaplastic stimuli than to fulfilling hematopoietic functions.

As implied later in the discussion of the pathology of osteopetrosis, evidence derived from the study of microscopic sections of marrow does not lend much support to the theory that a normal or hyperplastic marrow is crowded out of existence by endosteal bone formation. Indeed, there is little enough convincing evidence that the spleen, liver or lymph nodes become centers of compensatory hematopoietic activity. Certainly the ectopic blood formation on a quantitative basis is not proportional to the increase in size of these structures.

PATHOLOGY

Bone changes

Morphologic studies of anatomical changes in the skeleton indicate that certain alterations are constant and more or less fundamental, while others depend more particularly on the degree of development or rate of progress of the disease. This is well brought out in the abstracts by McCune and Bradley[13] of pathologic data reported in nineteen cases, sixteen of which were verified during life. Growth in length and diameter is slightly retarded; expansion and clubbing of the ends of the long bones, particularly the femurs, is characteristic. With but few exceptions evidence of periosteal involvement is lacking. Centers of

ossification appear about the usual time, and epiphyseal unions are but little delayed. The increased density advances from the diaphyses and becomes homogeneous except for bands and striations which parallel the epiphyseal lines. These alternating bands of increased and decreased density are pathognomonic of osteopetrosis and unquestionably reflect profound changes and intermittency in endochondral growth, osteoblastic production and trabecular resorption.

FIG. 1. PATIENT J. B. AT THE AGE OF 2 YEARS

One year later the liver margin extended nearly to the umbilicus and the splenic border to the umbilicus and brim of the pelvis.

Despite the hardness and implied durability in the name "marble bones," the fragility and tendency to fracture is actually increased, so that the name "chalky bones" was suggested by Pirie.[7] Single or multiple fractures may occur at any age from a few months to adulthood. In many of the reported cases, the diagnosis was quite unsuspected until after fracture had occurred. The line of fracture is characteristically at right angles to the shaft, and corresponds to the transverse bands of lessened density.

As a rule these fractures are not painful and unite promptly, although exceptional instances of protracted pain and delayed union have been noted.

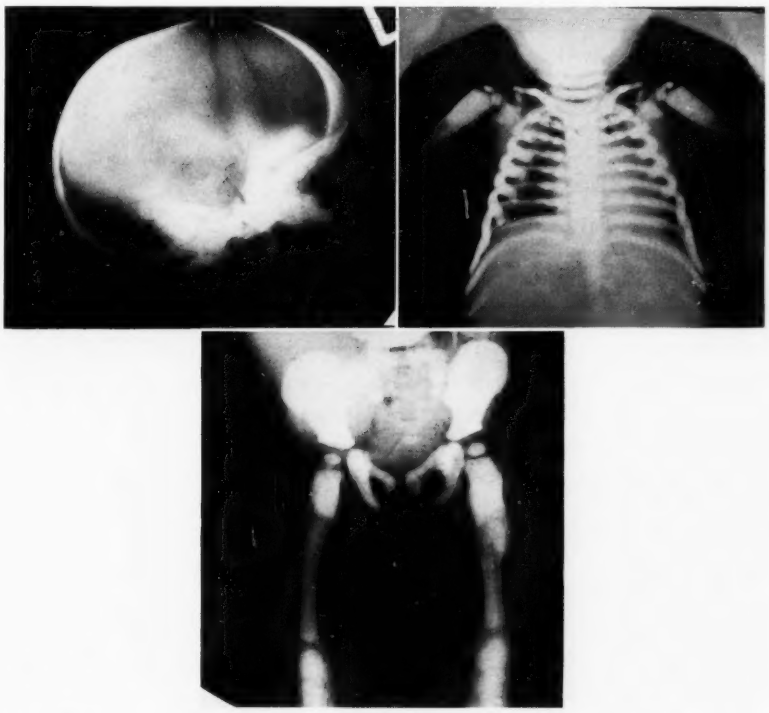

FIG. 2. ROENTGENOGRAM OF THE HEAD, TORSO, PROXIMAL ENDS OF THE HUMERI AND FEMORA SHOWING THE DISTRIBUTION OF THE INCREASED DENSITY OF THE SKELETON

Note the clubbing of the humeri and femora, and the opaque shadows of the base of the skull, ribs, and pelvic bones. Note the healed transverse fracture of the right femur.

Cranial bone changes are more extensive and of much greater importance in the basal portion. Ossification of the sutures is retarded and hydrocephalic changes have been observed. A deformed sella turcica may encroach on the pituitary gland, and result in disturbances of growth. Pressure on the optic nerves due to narrowing of the optic foramina leads to primary optic

atrophy. Nasal congestion and hemorrhage may be traced to pressure on the ophthalmic vein; nystagmus has been observed

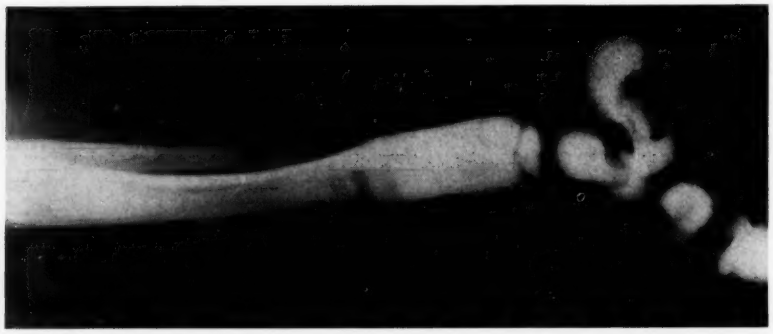

FIG. 3. ROENTGENOGRAM OF THE RIGHT LEG AND FOOT

Note the expansion and increased densities of the epiphyseal ends compared with the diaphysis, also the transverse striations parallel to the epiphyseal line.

FIG. 4. ROENTGENOGRAM OF THE LEFT FOREARM AND HAND

Note the clubbing and increased densities of the ends of the bones, also the transverse striations of alternating densities.

due to changes in the bony labyrinths; and distortion of the orbital cavity and venous congestion may cause exophthalmos. Constriction of the bony canals of the alveolar arteries has been

FIG. 5. A TYPICAL SECTION OF LIVER
Numerous fibroblasts. No evidence of new blood forming centers

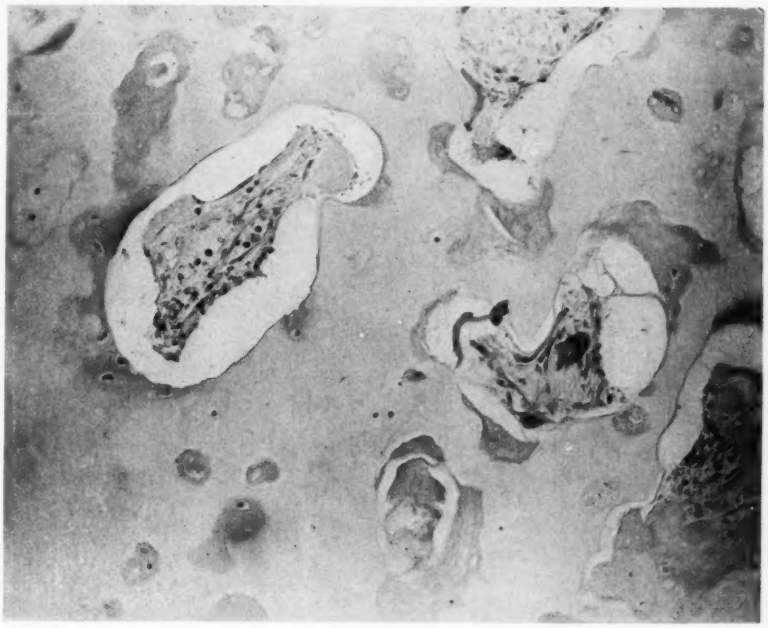

FIG. 6. LAIDLAW STAIN OF A SECTION FROM THE SPLEEN (LOW POWER)
Note the coarse reticulum

ascribed as the cause of dental caries and chronic suppuration of the mandible.

The nature of the increased bone density becomes evident in a study of microscopic sections. The lamellae of the cortex are increased in number and are more compact. The spongiosa presents a marked increase in the number and thickness of the individual trabeculae in which there is also a persistence of calcified cartilage. In many of the bone sections from our case the spongiosa is quite indistinguishable from the compacta.

Opinions differ as to the manner of production of these defects. Some have reported evidence of increased osteoblastic activity, others have interpreted their findings as evidence of diminished or abnormal osteoblastic production. Similar differences have been reported in the number and character of osteoclasts, in the degree of trabecular resorption and in the question of metaplasia from fibrous to osteoid tissue.

Hematopoietic system and blood changes

The important sequel to the great increase in number and size of the trabeculae is the contraction in size and obliteration of the marrow spaces and Haversian system. There is a marked reduction, of course, in the total amount of marrow and it was at first thought (Laurell and Wallgren,[12] Lorey and Reye[20]) that this decrease was the basis for the severe myelophthistic anemia, and that the enlargement of spleen, liver, and lymph nodes was simply an expression of compensatory hematopoiesis in extramedullary blood forming organs. Histologic studies of these structures, however, have shown a surprising lack of blood-forming activity. The reciprocal relationship that might be expected (on a basis of compensation) between the degree of bone change, the degree of anemia, and the evidence of hematopoietic activity is lacking. It has been suggested,[19] therefore, that osteopetrosis is not a distinct bone disease entity, that is, a disease sui generis, but rather a disease entity affecting the common progenitor of the hematopoietic and osseous systems, namely undifferentiated mesenchyme.

McCune and Bradley,[13] in their extensive review, conclude

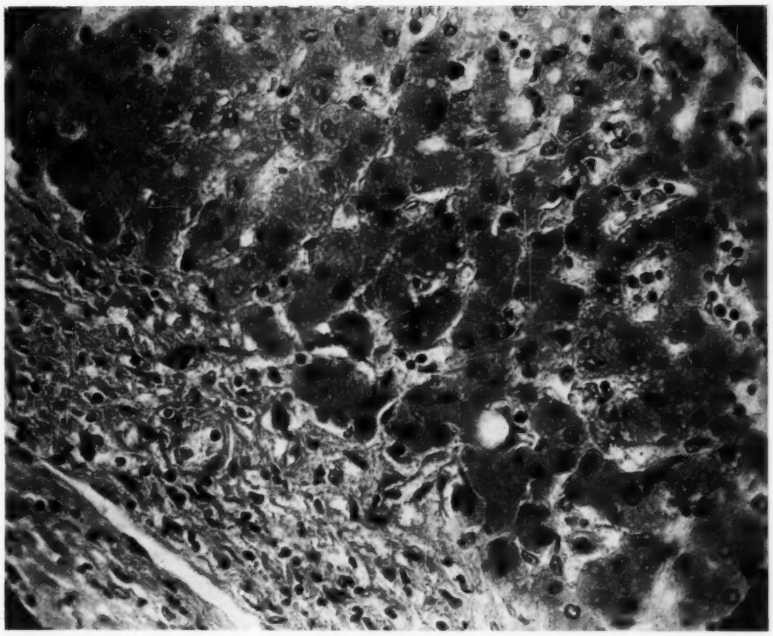

FIG. 7. LOW POWER MAGNIFICATION FROM THE SPLEEN
Note the coarse reticulum and absence of blood forming centers

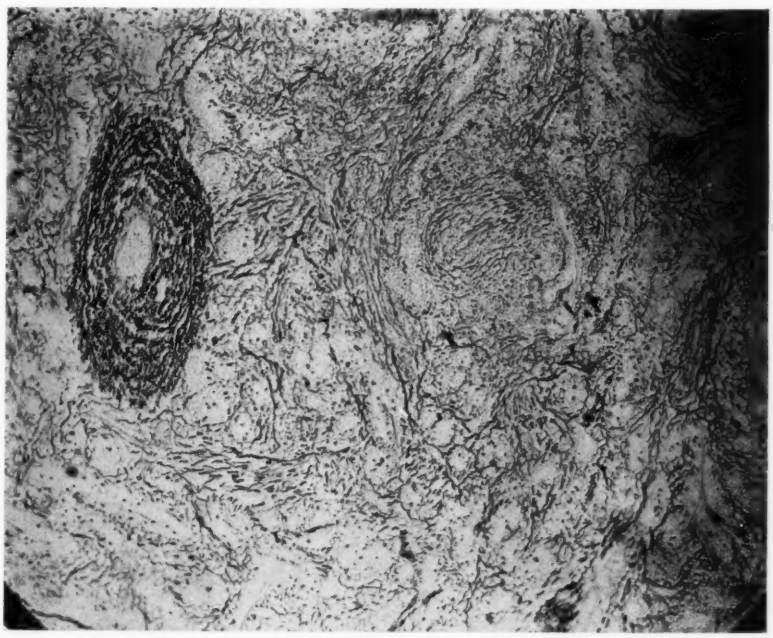

FIG. 8. ANILIN STAIN OF A SECTION OF SPLEEN
The coarse fibers on the right form the margin of one of the trabeculae

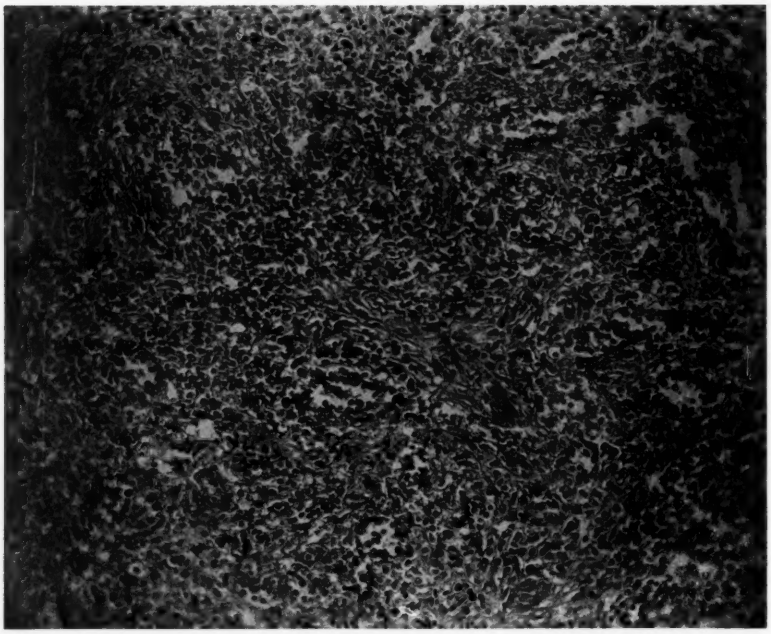

FIG. 9. LOW POWER MAGNIFICATION OF A SECTION FROM THE RIGHT TIBIA SHOWING THE INCREASED WIDTH OF THE TRABECULAE AND THE DISTRIBUTION OF THE FIBROUS MARROW

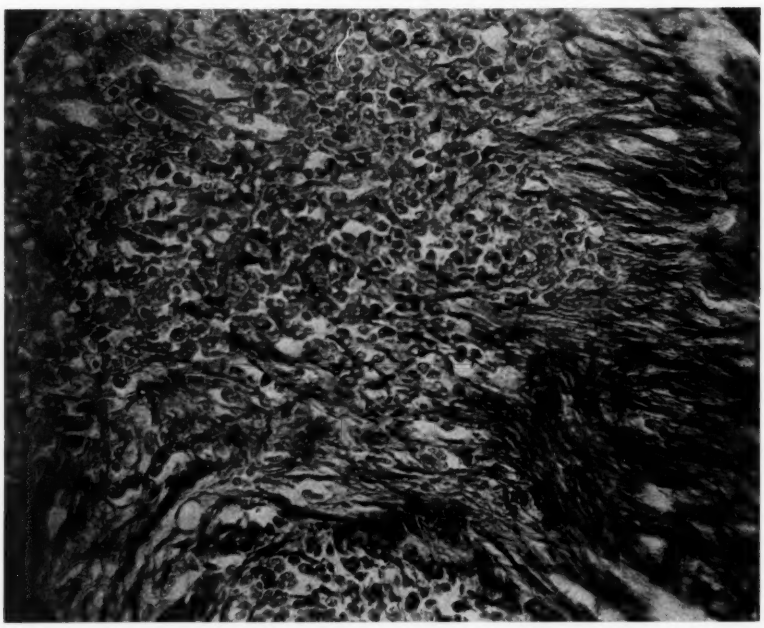

FIG. 10. HIGH POWER MAGNIFICATION OF A SECTION FROM THE RIGHT TIBIA

Note the persistence of calcified endochondral tissue, and scarcity of osteoid tissue.

that analyses of the chemical composition of bones reveal no constant or striking alterations in their calcium, phosphorus, magnesium, or carbonate content. The trend of blood calcium values as reported has been slightly high, while blood phosphorus levels have been slightly low.

As a rule the peripheral blood picture, as recorded in case reports, conforms to that of hypochromic anemia though the terminal stage may simulate aplastic anemia. The reduction in red cells and hemoglobin is frequently accompanied by the presence of a few immature erythrocytes and leukocytes.[13] Blood platelet estimations have varied from 350,000 and 180,000 per cubic millimeter (Kopyloff and Runawa[9] to a thrombocytopenia of 20,000 (McCune and Bradley.[13]) Determinations of color, volume, saturation, and icterus indices, also those of fragility, sedimentation, bleeding and clotting time have seldom been reported.

COMPARATIVE PATHOLOGY

The position of osteopetrosis in relation to other diseases with generalized manifestations in the osseous system is noteworthy. Morse[21] has grouped these disorders on a basis of the primary defects. Although not specifically included by Morse, it is evident that osteopetrosis should be placed with osteogenesis imperfecta (fragilitas ossium), recently regarded as an hereditary clinical entity,[29] in which the primary defect is in the mesoblast. While there is a failure of osteoblastic forming cells to lay down sufficient connective tissue ground work in these disorders, the exact reverse condition seems to obtain in osteopetrosis; that is, an over production of connective tissue ground work results in endosteal thickening and increased density. In contrast to these instances of primary mesoblastic defects may be cited rickets and osteomalacia where the primary defect lies in disorders of calcium absorption or fixation, or again in the bone malacia of renal rickets where the primary defect is calcium leakage with phosphorus retention.

DIAGNOSIS

The roentgenographic finding of opaque bones, either by accident or in connection with pathologic fractures, has usually

furnished the first diagnostic clue. In children, the combination of anemia, retarded growth, enlarged liver, spleen, and lymph nodes should arouse suspicion. When these findings are accompanied by pathologic transverse fractures and visual disturbances the clinical picture is complete. That there is an increasing interest in the subject is evidenced by the fact that more cases have been reported in the past seven years than in the twenty-six years between 1904 and 1930.

Occasionally, in leukemia there develops a diffuse sclerosis of bones, such as was first described by Heuck[27] in 1879 and since then by Newmann,[22] Von Jaksch,[23] Schwartz,[24] and Askanazy.[25] These leukemic changes are primarily infiltrative and usually involve the periosteum. Roentgenographic shadows are less opaque than in osteopetrosis, and the characteristic epiphyseal stratification with obliteration of the bony structure is lacking. Under the title "osteosclerotic anemia," Chapman[28] has reported two cases in which roentgenograms showed a diffuse moderately increased density due to an increase of connective tissue or trabeculae in the marrow space, leaving the cortex still recognizable. There was a marked myelophthistic anemia, but no fractures, no transverse striations, and no hereditary element.

In contrast to these examples of diffuse condensation of bone, cases have been reported of disseminated, localized foci of increased density under the names "osteopoikilosis" and "osteopoicilia" and characterized by spots on wavy striations of increased opaqueness. Sclerosing changes affecting single, individual bones have been described, such as the eburnizing osteitis of Putti,[26] also affecting groups of bones, such as the condensing osteitis of Sicard.

TREATMENT

If, as seems likely, the cause of osteopetrosis is correctly ascribed to an inherently abnormal histogeneis, that is, a perversion in mesenchymal development, the condition can not be cured. Therapeutic measures designed to modify the mineral and vitamin metabolism, or endocrine disturbances are quite futile. Splenectomy is contra-indicated. Fractures usually respond to orthopedic management, and blood transfusions may prolong life.

CASE REPORT

At birth this female child, J. B., was a footling presentation, weighed 7 pounds, and cried and breathed spontaneously. At six months she weighed approximately 12 pounds. Defective vision was suspected soon after birth, but owing to financial difficulties the child was not adequately cared for until after six months of age. When she was one year old roentengenograms of the skull and skin showed increased bone density.

At the age of two years the child was admitted to the Pediatrics Department of the University Hospital, Iowa City, Iowa, Nov. 17th, 1935 in the service of Dr. Jeans. The following is an abstract of the history, and clinical and laboratory findings for which the authors are indebted to the above mentioned service.

Entrance complaint. Blind since birth or early infancy; stomach trouble and constipation of 22 months duration; delayed teething; inability to walk.

Family history. The father and mother are well. One brother aged 3 years is apparently well; one brother aged one year is also well. Consanguinity is present in the family: the patient's great-grandfathers were brothers.

Medical history. She was bottle fed without cod liver oil or fruit juice during the first six months. At about 3 months of age the child was examined by an oculist who reported that she was blind and probably had hydrocephalus. After six months of age the child received orange juice, 15 drops of viosterol daily, and Pablum was added to a pasteurized milk formula. The first tooth appeared at fifteen months. Efforts were made to teach the child to walk, but at 2 years she was unable to support herself. At 18 months she repeated a few simple words.

Present illness. Since the child was 2 months old her eyes have rolled about, seemingly without focusing or coördination. She seemed to have light perception. The weight had remained stationary for the past month at 23¾ pounds. There has been an increasing fullness and distention of the abdomen.

Physical examination reveals an undernourished, pale, fussy, white female child of about 2 years unable to pull herself into a sitting position. Her forehead is strikingly prominent. The occipto-frontal circumference is 49 cm. The skin and mucosae are very pale. The patient seems hypersensitive to noise. The pupils are round, regular, and equal, but there is no response to light. There is a coarse rolling motion of the eyes, fairly well coördinated. The nose is small, bridge depressed, septum intact, and breathing noisy due to glairy mucus in the nares. The hard palate has a high arch; there are two incisors above and two below all showing evidence of dental caries. Mucosae of the mouth and throat are very pale. There is a chain of small glands in each side of the neck. The walls of the thorax are thin but symmetrical; with no notable deformities. Downward movement of the diaphragm is inhibited due to fullness of the abdomen. Auscultation, percussion, and breath sounds are clear.

Abdomen. On palpation a large, firm, smooth mass with rounded border

fills the whole left side extending down to the left iliac crest. The liver extends 4 cms. below the right costal margin in the nipple line. No abnormalities are observed about the genitalia or rectum.

Extremities. The ends of the humeri and femora are palpably enlarged. The deep tendon reflexes are equal and active. The circumference of the chest at the nipple line is 44.5 cm. The child's height is 79 cm., the sitting height 52 cm.

Routine laboratory examination. The urine examination, Wassermann, tuberculin, and undulant fever tests were negative. The red cell count was 2,500,000; the white cells varied from 6,200 to 11,050, the hemoglobin readings from 42 to 48 per cent. Examination of blood films revealed no abnormal leukocytes. The spinal fluid pressure ranged from 20 to 35 mm. Hg. The globulin, Meyers, and Wassermann tests were negative. There was 1 lymphocyte per cubic millimeter.

Roentgenograms. X-ray films of the cranium showed the tables to be of usual thickness. All bones of the skull were very dense with complete loss of trabeculation. The long bones presented an unusual appearance. The distal ends were enlarged and cylindrical but connected by normal sized shafts. There was an old healed transverse fracture of the right femur at the junction of the upper and middle thirds. The lower ends of the tibiae, ulnar bones, and radii show alternating transverse striations of greater and lesser density. Attempts to show the optic foramina were not successful.

Special laboratory examinations. November 21, 1935, the hematocrit reading of the blood was 20 per cent, the hemoglobin 5.14 grams per 100 cc., and the reticulocytes 3.6 per cent. The blood calcium determination was 12.7 mgm. and the blood phosphorus 4.5 mgm. per 100 cc. The blood phosphotase determination was 11.3 units, the icterus index 8.0 units, the bilirubin 0.4 mgm. and the VandenBergh delayed direct. These examinations were repeated November 25, 1935 with the following results: blood calcium 13.0 and blood phosphorus 3.7 mgm. per 100 cc.; the phosphotase 9.9 units; the icterus index 8 units; the VandenBergh delayed direct, urobilin 0.6 mgm. per 100 cc.

After three weeks observation the patient was discharged with a diagnosis of osteopetrosis. Dietary and therapeutic instructions were carefully followed, and the child continued to gain weight until September 1936. A blood examination October 27th, 1936, revealed the following: R.B.C., 2,180,000; W.B.C., 18,700; Hgb, 3.76 grams per 100 cc. Differential: Lymphocytes, 40.0; segmented, 24.0; staff, 15.0; juvenile, 6.0; myelocytes, 15.0. Many normoblasts and an occasional megaloblast present, marked poikilocytosis and anisoctosis.

NECROPSY

External examination. The body of a white female child aged 3½ years 83 cm. long, 46 cm. in circumference at the nipple line and 53 cm. at the umbilicus. The skin is waxy, white, of fine texture and without body hair. The anterior fontanelle measures 2 x 2 cm. The left pupil measures 4 mm. in

diameter, the right pupil 3 mm.; the sclerae are pearly white; the conjunctivae extremely pale. The mucosae of the mouth and nose are pale, and covered with a fine reddish foam.

The abdomen contains 100 cc. of clear straw-colored liquid. The omentum is free but devoid of all but traces of fat. The lower border of the liver is 9 cm. below the costal margin in the right nipple line, and 5 cm. below in the left nipple line. The spleen extends down to the brim of the pelvis, fills the left flank, and extends well up under the diaphragm. The other viscera are normal in size and position. There are no adhesions and the peritoneum is smooth and shiny; the mesentery is studded with pea-sized, sago-like nodules.

The spleen measures 17.5 x 9 x 7.5 cm. The surface is smooth, light gray, and homogeneous. The organ is firm and cuts with some resistance; the cut surface is wet, purplish gray in color and homogenous. The hilar portion of the spleen is filled with large firm nodular masses which extend to and merge with the tail of the pancreas. On section these nodules, 1 to 1.5 cm. diameter, are moist, shiny, and reddish gray in color.

The liver measures 20 x 12.5 x 7 cm., is firm, does not flatten out on the table, and presents no gross abnormalities. The cut surface is moist, and light brown in color with a fatty sheen. The lobulations are indistinct. The hepatic veins are considerably enlarged.

The pancreas measures 10 x 2.5 x 1.5 cm., it is of normal consistency, pale, moist, with distinct lobules.

The left kidney measures 7.5 x 4 x 3.5 cm. The fatty capsule is very scant, the fibrous capsule strips easily. The fetal lobulations are distinct, and the outlines between the cortex and medulla are well marked. The cortex averages 2 to 3 mm. in thickness. The cut surface is wet, the pelvis clean, and the calyces appear normal. The left adrenal weighed 3.5 grams and seemed to be normal. The right kidney and adrenal are similar to the left.

The left lung measures 15 x 8 x 5 cm. There are no airless areas, adhesions, anomalies, or deformities. A fine gray foam can be expressed from the bronchi. The cut surface is wet reddish gray and mottled in appearance and as it expands a fine foam exudes. The hilar nodes are pea-sized, firm, and gray. The left pleural cavity contains 300 cc. straw colored liquid slightly blood-tinged.

The right lung is similar to the left. The cut surface is very wet and drips with reddish gray foamy liquid. The right pleural cavity also contains about 300 cc. of liquid. Sections from both lungs float in water.

The heart measures 7 x 6 x 3 cm. The pericardium contains 30 cc. clear straw colored liquid. The epicardium is uniformly smooth and moist, the myocardium soft and flabby. Hydrostatic test of the aortic valve is normal. The other valvular orifices and valve flaps, the chordae tendineae and papillary muscles are normal.

The thymus remnants weigh 4 grams, are soft, edematous, somewhat mucoid in character.

Bones. The distal ends of the femurs, tibia, and proximal and distal ends

of the humeri are palpably enlarged. The periosteum is of normal thickness and texture. The bones and surfaces of the bones are very hard, and have a homogeneous grayish pink color. Resistance to sawing is very noticeable, especially in the outer two millimeters of bone.

Microscopic examination of sections from the right tibia and sternum shows a marked increase in the number and thickness of the trabeculae. The cortical lamallae are exceedingly compact. There is such a marked distortion of the elements that it is quite impossible to distinguish clearly between the compacta and spongiosa. There is a persistence of calcified cartilate and some callous formation. The intertrabecular spaces are filled with a cellular fibrous tissue in the interstices of which there are cells, occuring individually and in groups, which are difficult to identify. Here and there are small clusters of stem cells, and a few groups of myeloid elements with eosinophilic myelocytes predominating. Intermediate and transition forms between stem cells and mature erythrocytes seem to be lacking.

Sections from the spleen show a diffuse fibrosis and dense reticular increase not unlike that seen in the spleen of Banti's syndrome.

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AN EXPERIMENTAL STUDY OF THE BIPHASIC VAN DEN BERGH REACTION*

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In 1916 Van den Bergh and Muller[1] published a discussion of the reactions which occur when the diazo reagent of Erhlich[2] is added directly to blood serum. In their paper, which appeared shortly after a description of a somewhat similar quantitative technique by Van den Bergh and Snapper[3], it was suggested that the type of the serum diazo reaction is of diagnostic value. A considerable literature concerning this qualitative test has accumulated during the last two decades. As this has been adequately summarized and discussed recently by Barron[4] and by Magath[5], it will not be reviewed at length here.

The results of the qualitative diazo test upon serum were divided by Van den Bergh and Muller into two groups an immediate (sometimes called direct) reaction, and a delayed (sometimes called an indirect) reaction. There seems to be little doubt that two such types exist, and that they are of considerable diagnostic significance, but there is a good deal of variation in the way in which they have been defined. In general, it may be said that when the reaction is immediate a development of color begins promptly (usually given as within 30 seconds or one minute) and reaches maximum values almost immediately (usually given as about one minute). When the reaction is characterized as delayed or slowly reacting, the color change begins after an interval of time described by different observers as one to five minutes, and thereafter progresses rather slowly so that maximum values are not reached until after 15 to 30 minutes have elapsed.

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In 1919 Feigl and Querner[6] recognized the fact that some reactions are hard to classify under either of these two headings, since in some sera a prompt development of color was followed by a gradual further increase in the intensity of the color. They called such reactions "biphasic." A recent text book[7] adds a fourth group by classifying the biphasic reaction under two headings, a prompt biphasic in which a slightly reddish color appears in one minute and deepens to a violet in five minutes, and a delayed biphasic in which the red color appears in one to twenty minutes and slowly develops into a violet shade. The majority of laboratory manuals, however, list only three types of reactions: the immediate, the delayed, and the biphasic[8].

It seems, therefore, that there is some difficulty in satisfactorily classifying the color reactions in the intermediate group. A correct study of sera giving these reactions is of considerable importance, because, in our experience, they occur in a fairly large number of cases of hemolytic jaundice with high bilirubin values, in cardiac jaundice, and also in liver dystrophies (catarrhal jaundice, cirrhosis, etc.) in which the bilirubin concentration is not very great. It seems to be commonly accepted that the difference between the immediate and the delayed reaction is due to some slight physicochemical difference in the nature of the bilirubin causing them, although the nature of this difference has not been determined. Are both of these bile pigments regularly present together when the reaction is biphasic? This seems to be a logical conclusion from the general understanding of the test, and has served as a basis of much interesting speculation in many articles upon the pathology of jaundice. If the explanation is always as simple as this why have some investigators found it necessary to subdivide the biphasic reaction into two types? Do these different types have different clinical significance, and if so, what is their probable meaning? Some recent experiments appear to help somewhat in answering these questions.

In these experiments bilirubin, prepared according to the directions of Soffer,[9] was injected intravenously into human subjects. Among the patients studied were included some who were presumed to be normal, some with

hypochromic anemia showing the very low bilirubin values often observed in that condition, and others with various types of clinical jaundice. In each instance, samples of blood were drawn before and after the injection. Bilirubin was quantitatively determined in the plasma of these specimens by the method

TABLE 1

NUMBER	CLINICAL DIAGNOSIS	AMOUNT OF BILIRUBIN INJECTED	TIME OF SAMPLE AFTER INJECTION	BILIRUBIN CONTENT	FIRST APPEARANCE OF COLOR	QUALITATIVE VAN DEN BERGH (DIRECT REACTION) TYPE OF COLOR CHANGE
		mgm.	*minutes*	*mgm. per 100 cc.*	*seconds*	
I	Hypochromic anemia	100	Before	Trace		No color in 15 minutes
			5	2.05	30	"Slow" color
			20	1.0	60	"Slow" color
		30	Before	Trace		No color in 15 minutes
			5	0.63	90	"Slow" color
		60	Before	Trace		No color in 15 minutes
			3	1.9	120	"Slow" color
II	Hemolytic jaundice	30	Before	2.8	150	"Slow" color
			5	4.05	15	"Slow" color
III	Catarrhal jaundice	100	Before	0.78	15	"Fast" color
			5	2.75	15	"Fast" and "slow" colors together*

By " 'slow' color" is meant a pink color reaching a maximum intensity in 15 to 30 minutes after diazotization; by " 'fast' color" one reaching a maximum in one to two minutes.

* A strong pink color, apparently like that formed in the control specimen, appeared immediately after diazotization, but was obscured by a yellow or orange tint. The pink color slowly increased in intensity, and the yellow color faded slowly. At the end of fifteen minutes yellow could no longer be distinguished. The pink color appeared to reach a maximum in about thirty minutes, at which time it was as intense as the color of the indirect (quantitative) test made upon the specimen.

of Thannhauser and Andersen.[10] In the qualitative diazo test, which was carried out in the usual way, the color of the tube containing the plasma and diazo reagent was compared with that of another containing plasma and salt solution equivalent to the amount of diazo reagent used. The time interval preceding the first development of any pink color was recorded, and in addi-

tion the further type of change was studied, i.e., specimens were watched to determine whether maximum intensity appeared to be attained within a minute or two or whether the increase was gradual.

The results of typical experiments are given in table 1. Those in group I were carried out upon a patient with hypochromic anemia. The plasma of this patient contained only a very faint trace of bilirubin which could not be measured with accuracy (about 0.1 mgm. per 100 cc.). No positive qualitative reaction was seen during fifteen minutes of observation of the control specimen. It will be seen in the table that, when the concentration of bilirubin was increased following the injection, there was a shortening of the time in which the color first appeared. In two specimens the color was detected in a minute or less. In no instance, however, was maximum intensity reached in less than fifteen minutes. These experiments in group I are typical of all those performed upon individuals who did not show an increased concentration of bilirubin in the blood. They agree quite closely with the *in vitro* experiments of Harrop and Barron.[11]

The next experiment (Group 2) shows the effect of increasing the concentration of bilirubin in the blood of a patient with congenital hemolytic icterus. When the concentration was increased by the injection of the bile pigment the time in which a color appeared in the diazotized plasma was very greatly shortened, i.e., was reduced to 15 seconds, a value which would be regarded as immediate in any description of the Van den Bergh test. However, the color development in the specimen drawn after the injection, as well as in the control one, was of the slowly progressive (delayed) type, for maximum values were not reached for at least twenty-five to thirty minutes.

The experiment given in Group 3 is typical of a number showing the effect of increasing the concentration of bilirubin in the plasma of patients recovering from liver dystrophy (acute catarrhal jaundice). In the control specimen, containing only 0.78 mgm. of bilirubin per 100 cc. of plasma, an intense color developed promptly after the addition of the diazo reagent. This apparently reached its maximum intensity within a minute or less. In the specimen containing the injected bilirubin in addition to the pigment already in the blood as a result of the liver pathology, there was an immediate appearance of a pink color, but this pink was obscured by a yellow color obviously due to the unaltered, slowly reacting injected bilirubin. This yellow color appeared to be wholly replaced by pink or violet at the end of about fifteen minutes. This in turn continued to increase gradually in intensity, and apparently to reach its maximum value after thirty minutes.*

In all these experiments, as already stated, the injected bilirubin was of the slowly reacting type. We were not able, even

* This combination of the two colors is in the opinion of the authors the same phenomenon which Elton observed in pathological sera and called a "golden accentuation."[12]

by greatly increasing the concentration of bilirubin present, to obtain the type of immediacy which is found in cases of liver dystrophy and obstructive jaundice: i.e., the color never reached maximum values within a minute or two. The time of initial appearance of color could be greatly shortened, but it was regularly 15 to 30 minutes before the maximum intensity was reached.

A graphic representation of the results of these experiments is shown in the chart. Line A represents the usual prompt direct reaction such as was obtained in the control experiment in Group 3. It is seen that the maximum intensity of color occurs within 1 to 2 minutes. Line B shows the type of reaction which was found after bilirubin was injected into this patient. It must be remembered that the total amount of bile pigment present here was about three and a half times greater than in the control (line A). The first portion (almost vertical) of this graph is due to the bilirubin naturally present while the latter part (more nearly horizontal) is due to the injected bilirubin. Line D represents the usual response of normal plasma and of plasma from patients with hemolytic jaundice. The color is not visualized for some minutes and then slowly increases in intensity. When the concentration of bilirubin was increased in these cases, as was done in the experiments shown in Groups 1 and 2 in the table, a reaction occurred such as is indicated by line C. It must be remembered again that the total quantity of bilirubin here is greater than that represented by line D (the corresponding control experiments). It can be seen that although the bilirubin reacts very slowly and gradually over many minutes an appearance of color can be detected within a few seconds after the diazo reagent is added because the amount of bilirubin present is large.

It seems to us that the data given in the table and illustrated by lines B and C in the chart represent two entirely different types of reactions, both of which would almost inevitably be classified as biphasic. In both types the color developed promptly and in both the further development of the color continued for a long period of time. In one instance, represented by line C of the chart, and shown in experiments 1 and 2 in the

table, only one form of bilirubin was present, for injected bilirubin acts exactly as does the pigment of hemolytic jaundice.* This type of biphasic reaction was produced simply by increasing the total amount of bile pigment present. The other type of reaction is illustrated by experiment 3 and line B of the chart. Here there were two distinct types of reacting pigment in the plasma. One, the immediate reacting pigment of liver dystrophy already present, and the other the injected bilirubin. This biphasic reaction should be identical with one arising from the simultane-

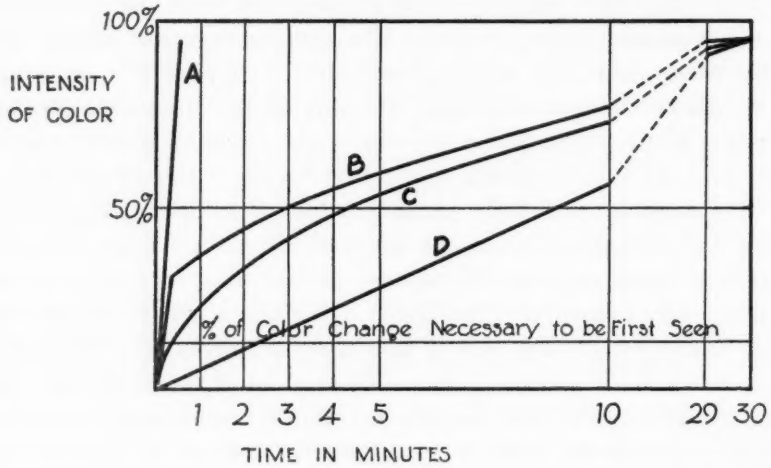

CHART 1. This chart, while not an exact mathematical representation of the results of the experiments, is a convenient way of illustrating them.

ous occurrence in the same patient of hemolytic jaundice and obstructive jaundice or liver dystrophy.

The question arises as to whether it would be possible to distinguish these two forms of biphasic Van den Bergh reactions from each other. It seems that it would be almost impossible to do this except when there is enough of the slowly reacting pigment present in a rather mild case of liver dystrophy or obstructive

*Not only does the injected bilirubin react with the diazo reagent in the same manner as does the pigment of hemolytic jaundice, but as experiments of the authors have shown, the chloroform solubility of these two pigments is identical.

jaundice, to give a distinct and unmistakable yellow or orange color to the reacting mixture in addition to the usual pink tint. If the immediate reacting pigment were present in high concentrations, or the delayed reacting form in low ones, they do not believe that it would be possible to decide with certainty which type of biphasic reaction was present.

SUMMARY

When the bilirubin in the blood stream of normal patients or of patients with hemolytic jaundice is increased by injecting alkaline solutions of bilirubin, the time in which a color appears after the addition of the diazo reagent to plasma is decreased. The general type of the reaction, however, does not resemble that seen in liver dystrophy and obstructive jaundice, for the color attains maximum values slowly. When the pigment is present in high concentration the results satisfy the definitions of a biphasic reaction. Another type of biphasic reaction can be produced by injecting bilirubin into a patient with liver dystrophy or obstructive jaundice with low bilirubin concentrations. In such patients the pathological pigment reacts rapidly and the injected pigment reacts slowly. It is sometimes, but not always, possible to recognize this biphasic reaction just described because the unaltered injected or the delayed reacting pigment present may give a yellow color which obscures the characteristic pink of the immediate Van den Bergh reaction by a yellow or orange color.

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BILATERAL CORTICAL NECROSIS OF THE KIDNEYS*

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Symmetrical cortical necrosis of the kidneys is a peculiar, rare disease occurring most commonly in pregnant women immediately after delivery although a few cases have been reported in men and children, in which quite obvious infectious diseases, such as scarlet fever, tonsillitis, diphtheria, tuberculosis and malaria, were followed by this very serious pathology of both kidneys.

If the disease occurs in pregnancy, the fetus is stillborn, usually with easy spontaneous delivery. Live birth was observed only in three instances, the cases of Jardine,[18a] Lloyd,[26] and Jardine and Teacher.[19]

Clinically, a variety of symptoms is reported. In many cases there was some evidence of toxemia of pregnancy before delivery. In others pain suddenly develops in the kidney region several hours after the delivery. Furthermore, there may be epigastric pain and tenderness, abdominal pain or distension and vomiting, headache, drowsiness, visual disturbances, vaginal bleeding and retroplacental hemorrhage. Convulsions may occur which may simulate eclampsia and Klotz,[25] Herzog,[17] Geipel,[12] Jardine and Kennedy,[20] and Kellar and Arnott[24] have noted the association of eclampsia with this kidney pathology. The patients are usually afebrile, feel fairly well, are relatively bright and often clear mentally, but in some cases are unconscious. The blood pressure may be elevated but is usually within normal limits. There is a progressive secondary anemia with a moderate leucocytic reaction. The blood chemistry shows retention of nitrogenous products. The NPN rises gradually and may reach 300 mgm. as in Dalrymple's[9] case. The creatinine shows a

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similar decided continuous rise. The face, feet and hands may become edematous.

The most important clinical symptom is anuria, complete or incomplete, the duration of which varies up to twenty days. The small amount of urine obtained in cases of incomplete anuria or during incompletely anuric periods, is loaded with albumen, red and white blood cells and with various casts. However, prior to the onset of the disease the urinary findings were entirely negative in the large majority of the published cases. Clinically, because of frequent complete absence of uremic signs and symptoms, the anuria resembles that seen in bilateral obstruction of the ureters.

Death usually ensues quite suddenly. A few cases have been reported as having recovered.

Pathologically, both kidneys present an almost symmetric and very characteristic picture. They are somewhat enlarged and the capsule strips with ease. The surface is quite mottled with alternating pale and red areas of irregular size and shape. On section the picture is striking. Almost the entire cortical substance, specifically the outer two-thirds, shows a pale yellow, putty-like discoloration due to necrosis, very similar to that seen in anemic infarcts. The necrosis extends down into the columns of Bertin. Just under the capsule there are areas with thin, seemingly unaltered, somewhat hemorrhagic strips of cortical tissue. The inner border of the necrosis shows a serrated edge and a hemorrhagic zone. About one-third of the cortical substance above the medulla seems to remain unaltered. There may be several strips of normal looking cortical tissue running up to the surface and alternating with necrotic cortical substance. These findings are responsible for the patchy appearance.

Symmetrical cortical necrosis of the kidneys was first described by Juhel-Rénoy[23] in 1885 in association with scarlet fever in a girl of 16. Since that time reports occur in the English literature, and occasionally in American publications.

Because of the uncertainty concerning the pathogenesis and etiology of this disease the present study and report seems warranted.

REPORT OF A CASE

The patient was a 33 year old white female who had had four previous deliveries and six miscarriages. Her last pregnancy was in 1928, during which the face and hands were edematous, mental and visual disturbances were noted, and there was albumen in the urine. In March, 1929 she had scarlet fever, but stayed in bed for only two weeks. There was no albuminuria at that time. During her recent pregnancy she had been seen once a month. The urine was negative for albumen and the blood pressure was normal. She was admitted to the Rochester General Hospital at 2:00 P.M. on February 27, 1930 bleeding profusely. The cervix was dilated two fingers breadth and the placenta was lying low within reach of finger. A No. 7 Vorhees' bag was inserted which was expelled and a stillborn fetus delivered at 2:30 P.M. of the same day. The uterus bled profusely and after manual removal of the placenta the uterus and vagina were packed with gauze. There were no lacerations. Because of the profuse hemorrhage 600 cc. of citrated blood was transfused. A catheterized urine specimen of less than 50 cc. was obtained. The urine was straw-colored, turbid, albumen; 4 plus, no sugar. The sediment showed occasional leukocytes, many red cells, and many hyalin and granular casts.

The vaginal and uterine packings were removed at 9:00 A.M. the next day. At 10:45 A.M. she had a severe chill lasting for 20 minutes and her general condition was only fair. She had not voided nor had any urine been obtained on catheterization. Fluids were forced. Examination of eye grounds showed nothing abnormal. On March 3 the patient had not yet voided. She was conscious, rational, felt fine, and slept a great deal. At night her extremities and abdominal muscles were spastic with hyperactive reflexes. A second transfusion of 500 cc. of citrated blood was given. Two days later the general condition remained about the same, the patient being conscious, rational and rather cheerful. The temperature was only slightly elevated. No urine had yet been passed.

On March 6 cystoscopy was done. There was no urine in the bladder and no urinary secretion from the catheter. The bladder and ureteral orifices were normal. A plain X-ray picture was negative for stone in the urinary tract.

On March 7 a small amount of urine was obtained after a complete anuria lasting for seven days and from March 7th until death a few ounces of urine were obtained daily by catheter.

On March 9 the patient had been very restless during the night, and during the day her condition was definitely worse and she was vomiting almost incessantly. Because her condition was becoming increasingly serious, surgical measures were attempted and decapsulation of the left kidney was performed, the operation being without difficulty, requiring twelve minutes.

On March 10, although clinically worse, about 90 cc. of urine was obtained

by catheterization. The patient was irrational at times. The CO_2 combining power equalled 21 per cent and sodium bicarbonate was given by mouth.

On March 11 about 60 cc. of urine was obtained by catheter. The patient was irrational and had an attack of extreme air hunger at noon. CO_2 combining power equalled 13 per cent. She was relieved by intravenous sodium bicarbonate, 75 grams of $NaHCO_3$ having been given intravenously in the last 24 hours. At night she appeared to be moribund. During the day her temperature rose up to 104°, although previously it ranged between 98° and 100°. The blood pressure rose slowly from 138/80 on March 1 to 152/108 on March 7, but dropped to 138/86 on March 11. Death occurred on March 12, 1930.

LABORATORY EXAMINATIONS

The very small amount of urine obtained daily by catheter from March 7 to 11 showed 2 to 4 plus albumen. RBC and WBC were abundant in the sediment and hyalin and granular casts were always very numerous. On one occasion the urine was composed of almost gross blood.

Blood counts. RBC = 4,120,000–4,000,000. WBC = 26,000 with 95 per cent polymorphonuclears on February 27, dropping to 13,200 on March 8. Hgb. rose from 55 to 76 per cent.

Chemical examination of the blood was done daily. On February 27, NPN 25 mgm.; uric acid: 2.7 mgm.; creatinine 2.6 mgm. These figures rose gradually each day and on March 11, one day before the patient's death, NPN 138 mgm.; uric acid: 15.5 mgm.; creatinine: 18.5 mgm.

The clinical diagnosis was: Placenta praevia, post partum hemorrhage, anuria, and uremia.

Autopsy was performed about ten hours after death.

POSTMORTEM EXAMINATION

A well developed and fairly well nourished young white female body showing moderate postmortem lividity and marked rigor mortis. There was a curved incision over the left kidney region with soft rubber tissue drains. The head was not opened.

Chest. The left pleural cavity contained about 300 cc., the right about 700 cc. of a serofibrinous exudate. The lungs were crepitant throughout with some hypostatic congestion and edema. The heart was slightly dilated. The walls were soft, friable, pale grayish red. All valves and the aorta were smooth. In the posterior mediastinum there was a fibrinous exudate in the soft tissue spaces.

Abdomen. About 100–200 cc. of serofibrinous exudate was found in the abdominal cavity. The *spleen* was enlarged, soft and on section pale grayish-red. The *liver* was soft and pale and on section quite mottled, with small scattered pale yellow areas. Both kidneys were slightly larger than normal, the total weight of both being 365 grams.

Left kidney. The capsule had already been stripped at time of the surgical

decapsulation. The surface was smooth but presented a mottled appearance because of grayish-yellow areas, of irregular shape alternating with similarly irregular darker red and hemorrhagic areas. On section the cortex was slightly swollen and showed the pale grayish-yellow discoloration of necrosis with jagged outline toward the pyramids and extending down into the columns of Bertin (fig. 1). The discoloration was very similar to that of the coagulation necrosis

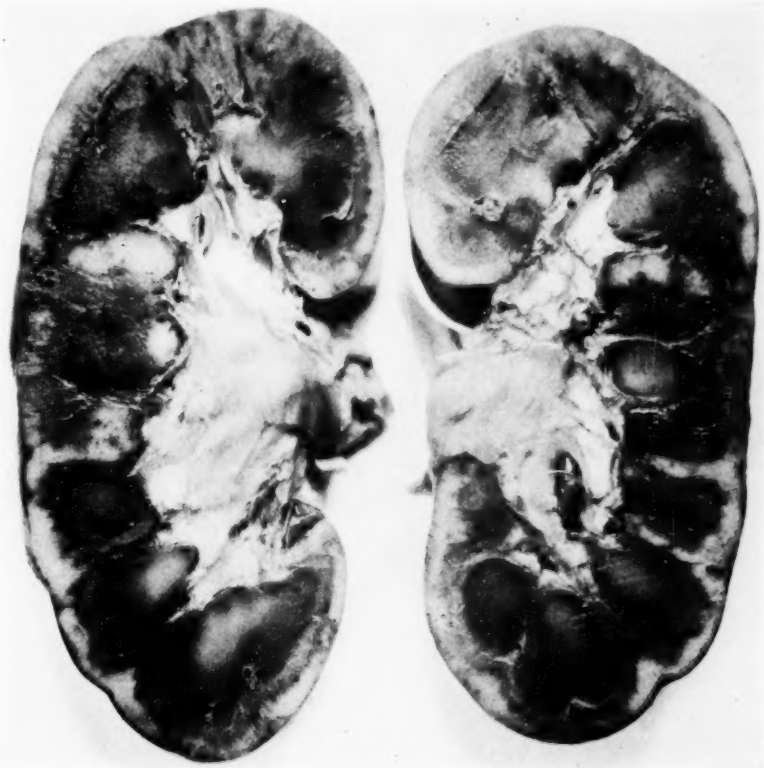

FIG. 1. PHOTOGRAPH OF GROSS SPECIMEN OF KIDNEY FROM THE REPORTED CASE

About $\frac{3}{4}$ scale

seen in anemic infarctions The necrosis was bordered against the deeper layers by a thin and irregular hemorrhagic zone. On first impression, this pathology seemed to involve the entire cortical substance, however, a close examination of the routine longitudinal surface and of numerous other sections made transversely (fig. 2) revealed that the necrosis was somewhat patchy in distribution, varying in thickness from 3 to 6 mm.

Between the necrotic zone and the pyramids there was a normal looking cortex, about 3 mm. in width. There were also many narrower and wider striations which run clear up to the surface and apparently formed of well preserved, although quite hemorrhagic, cortical tissue. The red mottling of the kidney surface was partly due to these areas. The necrosis usually reached to the surface, especially when it involved a wide surface area. In other places there was a very narrow rim of seemingly normal, but sometimes hemorrhagic, cortical tissue just beneath the capsule. As figure 2 demonstrates, this zone

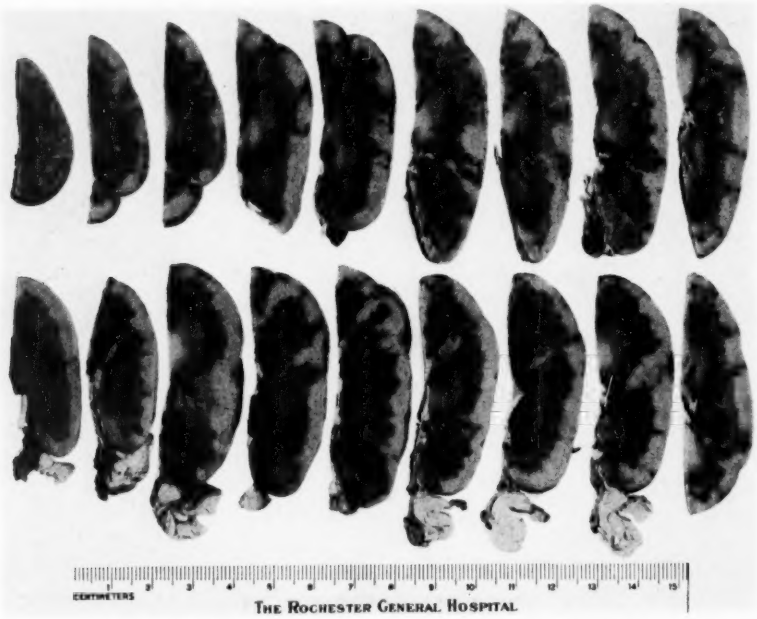

FIG. 2. TRANSVERSE CUTSECTIONS OF THE KIDNEYS

The slightly patchy nature of the cortical necrosis is more evident than on a single cutsection.

is not present as a regular feature. The medullary substance was grayish-red and showed no remarkable pathology. The arcuate and interlobar arteries did not present occlusion of their lumina. There were thrombi, however, parietal or occluding, in a few of the arcuate and interlobar veins and in one of the larger renal veins.

The right kidney showed essentially the same changes as the left but without decapsulation. Stones were not found in the kidney pelves or ureters.

The bladder contained no urine and the mucosa was moderately injected. The adrenals, pancreas and gastro-intestinal tract were grossly normal.

The uterus was the size of a grapefruit and its entire cavity was covered with a yellowish green coating On section, this was seen to involve only the surface, while the veins of the wall were all thrombosed. The right ovarian plexus and right ovarian vein were greatly distended and filled with thrombi. The thrombus of the right ovarian vein almost reached the inferior vena cava. The thrombi were softened, grayish pink and covered only by a thin shell of compact thrombus.

The parametrium, broad ligament, left ovarian plexus and vein showed no pathology.

ANATOMICAL FINDINGS

(1) Septic puerperal endometritis, thrombophlebitis of uterine veins, purulent thrombophlebitis of right ovarian plexus and of right ovarian vein. (2) Septicemia, Septic splenitis. (3) Coagulation necrosis of the greater part of the cortical substance of both kidneys (Symmetrical cortical necrosis). (4) Bilateral serofibrinous pleuritis, serofibrinous peritonitis and fibrinous posterior mediastinitis.

BACTERIOLOGIC EXAMINATION

Direct smear of the exudate of the uterine cavity showed Gram-positive diplococci, some in short chains, and Gram-positive and negative bacilli. Direct smear from right ovarian vein revealed only Gram-positive diplococci. Culture of the uterine exudate showed non hemolytic streptococci and staphylococcus albus. Culture of the thrombus from the right ovarian vein, however, presented a pure growth of very markedly hemolytic (Leta type) streptococci.

HISTOLOGIC EXAMINATION

Heart. Marked fatty degeneration. Lungs. Moderate edema and passive congestion with hyaline thrombi in a few small vessels just beneath the pleura. Posterior mediastinum. Fibrinopurulent inflammatory reaction. Spleen much congested. The sinuses are distended and filled with red cells and in many sinuses there are groups of large cells not unlike hemopoetic foci. There are numerous leucocytes in various parts of the pulp.

Pancreas, adrenal glands. Moderate passive congestion. Liver: central necrobiotic areas with much fatty degeneration and with a moderate accumulation of leucocytes. Moderate passive congestion.

Uterus. Inner surface is covered by a thick fibrino-purulent exudate and necrotic material, in which masses of bacteria are evident. The veins of the uterine wall are much dilated throughout and filled with purulent and ordinary thrombi, occasionally with signs of organization. Several of the thrombi contain masses of bacteria. The lymph vessels are empty. The uterine wall is edematous and slightly infiltrated with leucocytes and lymphocytes. All veins of the right ovarian plexus show purulent thrombi which contain masses of bacteria. Bacterial emboli of capillary vessels were observed in various organs

(lungs, liver, adrenals and spleen). The kidneys also showed bacterial emboli, but only in the capillaries of the non-necrotic areas and in the medulla, with no evidence of inflammatory reaction.

Kidneys. Both kidneys present the same histologic picture. In the broad necrotic zone the kidney tubules have lost their usual structure and their nuclei have completely disappeared (figs. 3, 5, 7). The lumina are either empty, or filled with various material, such as greatly swollen and necrotic tubular epithelium, amorphous granular debris or, toward the peripheral parts, with

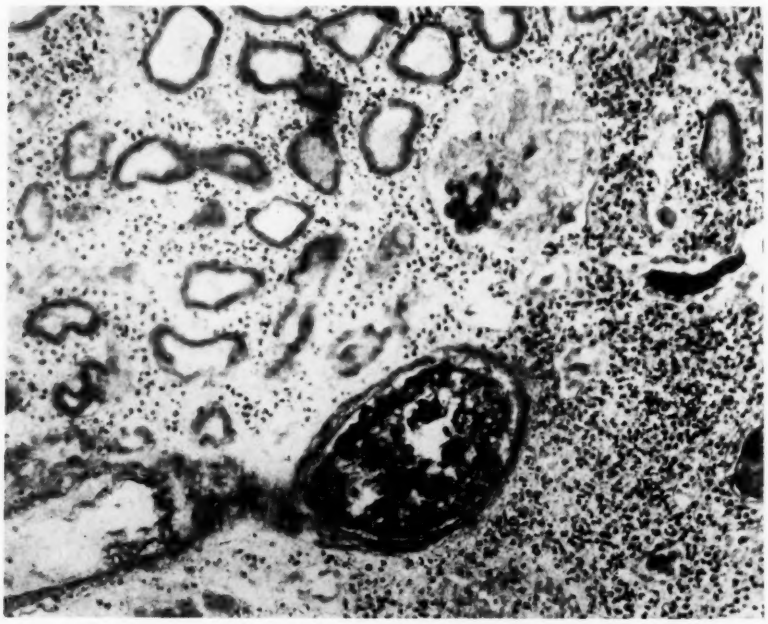

FIG. 3. MARKED LEUCOCYTIC REACTION IN THE NECROTIC ZONE
Magnification × 180

abundant red cells. Occasionally a well stained leucocyte is found in the lumen. The outline of the necrotic basement membrane is visible.

The interstitial connective tissue is also necrotic. Nevertheless, moderate numbers of leucocytes are found throughout and some areas are virtually packed with leucocytes (fig. 3). Many of the leucocytes are well stained, others are apparently disintegrating. At the periphery of the necrotic zone, the leucocytic infiltration is a very irregular feature and often absent. Scattered hemorrhages, at times covering large areas, are visible, especially at the periphery where they seem to merge with those of the adjacent normal cortical substance.

The arterial system of the necrotic zone, namely the glomeruli, afferent and

interlobular arteries, presents a great dilatation and thrombosis. The glomeruli are necrotic, but the outline of Bowman's capsule and that of the glomerular capillaries is clearly discernible. The capsular space is usually gone and markedly distended glomerular loops are filled with abundant laked, hemolyzed red blood corpuscles, among which more or less well stained normal ones may be visible (fig. 4).

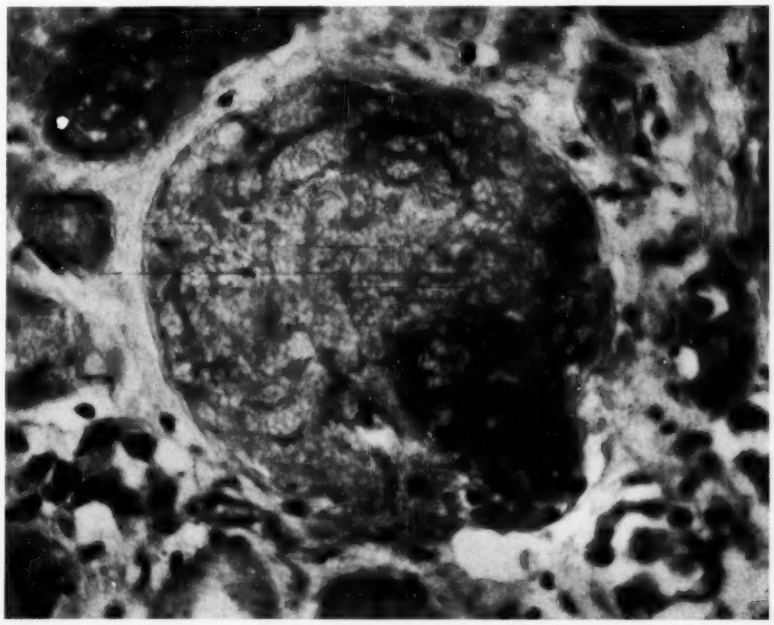

FIG. 4. GLOMERULUS FROM THE EDGE OF THE NECROTIC ZONE

Greatly distended and engorged glomerular tuft. The engorgement is due to red blood corpuscles, which are stained very poorly, only their outline being visible. There are also two hyalin thrombus masses located probably in the afferent and efferent arteries. Magnification × 400.

Leucocytic infiltration may be present in some of the glomeruli. Hyalin material, the so called hyalin thrombus, is found in some of the loops in many of the glomeruli. Either this hyalin thrombus fills the lumen completely, forming a perfect mold of the capillary, or the hyalin material is arranged in a tubular fashion, thus leaving a small central lumen.

The afferent arteries can be studied in almost any of the abundant sections prepared from various parts of the kidneys. Practically all afferent arteries are plugged by a seemingly compact hyalin thrombus mass (fig. 5), often retracted from the wall. On cross section, a tubular arrangement of the hyalin

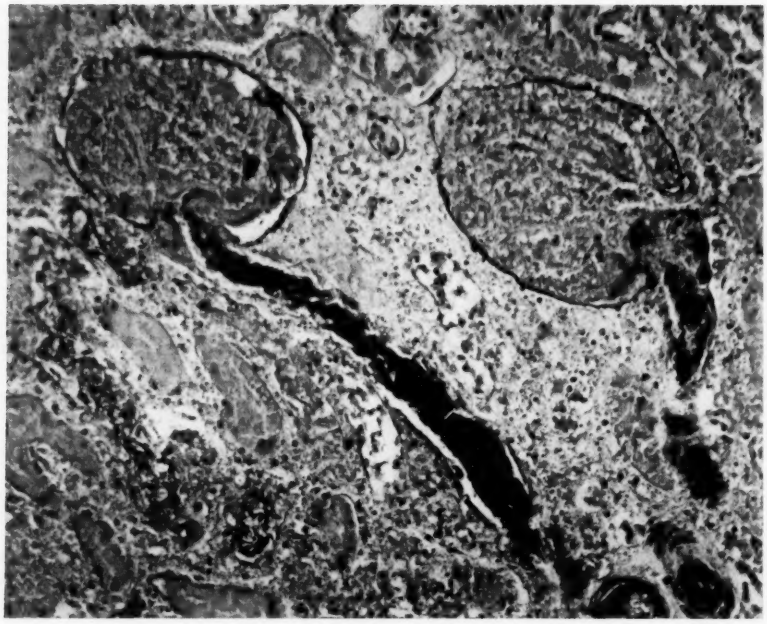

FIG. 5. AFFERENT ARTERIES PLUGGED BY A SEEMINGLY COMPACT HYALIN THROMBUS MASS

Magnification × 180

FIG. 6. GENERAL STRUCTURE OF THROMBI IN THE INTERLOBULAR ARTERIES

Magnification × 28

mass may be obtained. The greatly distended and thrombosed interlobular arteries are seen cut either lengthwise or transversely.

An interlobular artery cut in its entire length shows in the distal half an arrangement of the hyalin mass parallel to the arterial wall, while the central lumen is filled with very pale, apparently hemolyzed red blood corpuscles. The proximal half shows irregularly piled up hyalin masses in the crevices of which there are well stained and conglutinated red blood corpuscles (fig. 6). In transverse sections the tubular character of the hyalin thrombus mass is quite

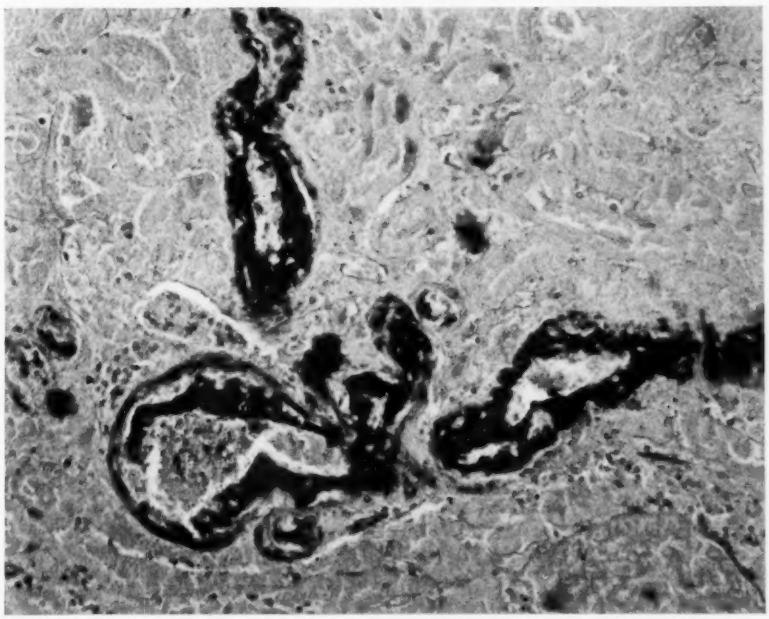

FIG. 7. INTERLOBULAR ARTERY AND AFFERENT ARTERIES SHOWING TUBULAR ARRANGEMENT OF THE HYALIN THROMBUS MASS

Gram-Weigert stain. Magnification × 180

obvious (fig. 7). The proximal end of the thrombus reaches somewhat below the necrotic area. In this end part of the thrombus there was occasionally definite evidence of organization. Below the thrombosed section of the interlobular arteries there seems to be somewhat of a collapse in much contrast to the distention noted above.

The wall of the afferent and interlobular arteries appears to be necrotic and some places of rupture are noticed. The hyalin mass is usually retracted from the wall, leaving a space which shows some distorted laked red blood corpuscles.

The hyalin thrombi of the glomeruli, afferent and interlobular arteries ap-

peared as somewhat glassy, bluish purple masses when stained with the routine hematoxylin-eosin method. With Gram-Weigert's stain for bacteria and for fibrin a lighter or deeper blue color was obtained. Bacteria could not be found in the thrombosed arteries. When the hyalin masses were stained only very light blue, it became evident that they were formed of an enormous number of hemolyzed, shriveled and broken down red blood corpuscles which were packed almost into a compact mass (figs. 8 and 9). Some white blood cells and fairly

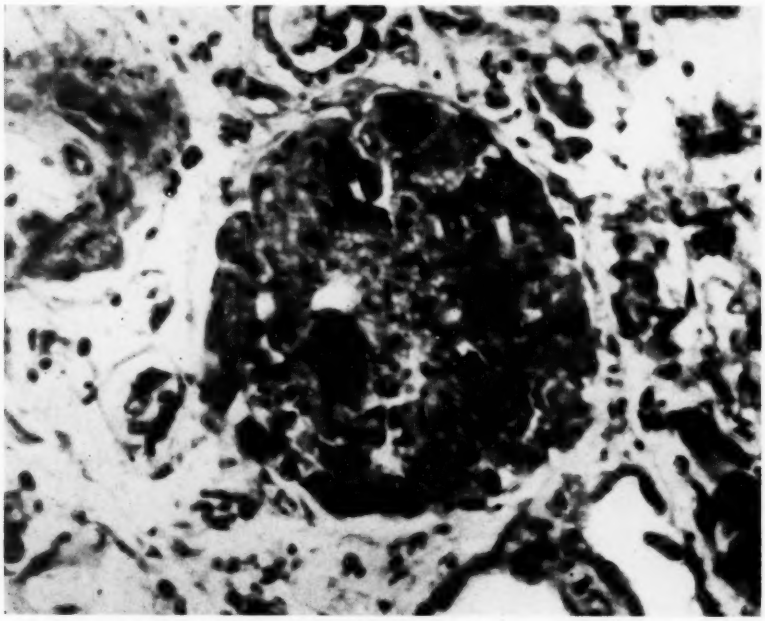

FIG. 8. GLOMERULUS LOCATED IN THE NORMAL CORTICAL TISSUE JUST ADJACENT TO THE NECROTIC PART

All glomerular loops are greatly distended by red blood corpuscles, a considerable part of which stains very poorly. Hyalin thrombus mass in one of the capillaries. Magnification × 400.

normal red blood corpuscles were present in the thrombi and the amount of fibrin was minimal. A few interlobular veins showed parietal thrombosis. The structure of this thrombus, however, was entirely different from that found in the arteries.

The very thin layer of undegenerated cortical tissue just under the capsule is formed mainly of tubules. On the other hand, the necrosis reaches clear to the surface in many areas, leaving untouched only a thin sheet of connective tissue just beneath the capsule.

The cortex below the infarcted area shows only one to three levels of glomeruli. The glomeruli immediately joining the necrotic areas usually show greatly distended glomerular capillaries filled with laked and well stained red blood corpuscles. The poorly stained or crenated red blood corpuscles become arranged close to the wall of the glomerular tufts, almost relining their wall. There may be hemorrhages into Bowman's capsule. Other glomeruli are somewhat collapsed, but may show red blood corpuscles and even hyalin thrombi in

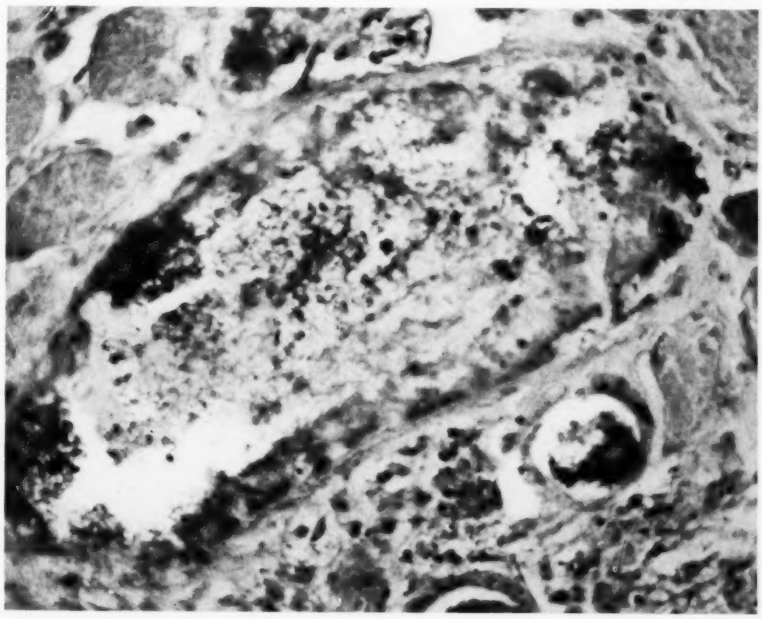

FIG. 9. INTERLOBULAR ARTERY SHOWING MASSES OF SHRIVELED, BROKEN RED BLOOD CORPUSCLES ARRANGED AT THE PERIPHERY

The tubular shaped hyalin thrombus mass has disappeared when the Gram-Weigert stain was well differentiated. An afferent artery is also seen in the picture. Magnification × 400.

some of the capillary loops. A few of the glomeruli seem to be without remarkable change. In many of the tubules there are various casts, such as hyalin, granular, cellular, leucocytic and RBC casts. Other tubules appear to be empty.

The grossly non-necrotic strips of cortical tissue show the glomeruli in a state similar to those found in the cortex just below the necrotic zone. In a few of these strips only some of the afferent arteries are plugged by hyalin thrombus

while the interlobular artery shows no thrombosis as yet (fig. 10). The capillary vessels are much distended and engorged and there are hemorrhages into the tissues. The non-thrombosed arteries contain abundant very pale and practically laked red blood corpuscles and among these a moderate number of well stained, apparently normal red cells. On the contrary, the capillary vessels between the tubules contain mainly well stained red cells.

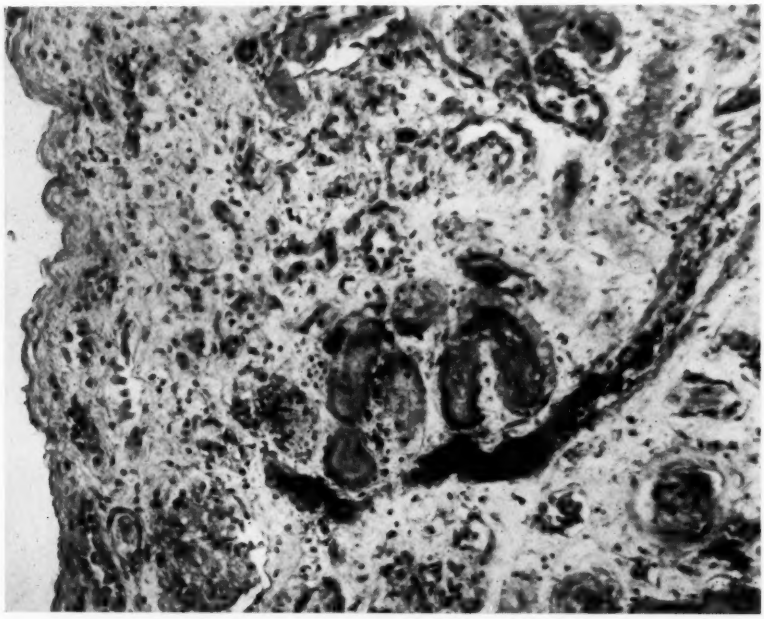

FIG. 10. AFFERENT ARTERY FROM A NON-NECROTIC STRIP OF CORTEX, SHOWING OBSTRUCTION AND DISTENSION BY HYALIN THROMBUS

The proximal part of the artery is not thrombosed and the wall shows no signs of necrosis. Magnification × 180.

The blood vessels of the other organs and the spleen were also examined for evidence of laked red blood corpuscles. Some of the blood vessels of the lung contained abundant hemolyzed red cells, but there were, however, only a few of these cells in the spleen and in the capillaries of the liver.

DISCUSSION

The foregoing study of a case of symmetrical cortical necrosis of the kidneys seems to fit in well clinically with those so far reported in the available literature. The gross pathology and

histopathology of the kidneys is also similar to the cases hitherto described. Nevertheless, there are differences in the finer histopathological changes and, to a certain extent, in their interpretation.

All authors agree that the plugging of the arteries is caused by thrombosis. Only Juhel-Rénoy,[23] reporting the first cortical necrosis during the course of scarlet fever, thought of emboli, "probablement embolies parasitaires," due to which glomerular filtration became arrested and the anatomical lesion of infarction was produced.

The structure of the thrombi was always carefully studied and its peculiar hyalin and tubular character reemphasized. It was thought that the thrombi were composed of fibrin and blood platelets, hence the hyalin, glassy appearance. Von Zalka[38] noted, however, that the amount of fibrin was very scanty, and furthermore that the thrombus masses did not show the structure typical of thrombi. Recently De Navasquez[28] reported on his study of twelve cases of cortical necrosis and on three cases of industrial dioxan (diethylene dioxide) poisoning causing cortical necrosis of the kidneys. He came to the conclusion that the intravascular masses are not true thrombi but consisted of conglutinated red and white corpuscles which have become pressed into a structureless hyaline mass with little or no fibrin formation or platelet deposit. The study of the hyalin thrombi of my case confirms De Navasquez's observations. Not only the hyalin tubular structures of the interlobular arteries, but also the hyalin thrombi of the afferent arteries and of the glomerular tufts were formed mainly of laked, shriveled, crenated red blood corpuscles, packed into an almost compact, dense, hyalin mass. The paleness in a majority of the red blood corpuscles, which seemed to be formed only of stroma and were practically devoid of hemoglobin deserves emphasis. The central parts of the thrombi were composed of similarly pale, although normal shaped, red blood corpuscles. Fibrin was absent or present in minimal amount. As to the beginning of thrombosis, Herzog,[17] Schüeppel,[33] and Glynn and Briggs[15] are of the opinion that the thrombosis begins peripherally and extends proximally. In

other words, it begins in the glomeruli and afferent arteries. Jardine and Kennedy's early case confirms this view. Here the cortical necrosis was slight, and the thrombi were found chiefly in the capillaries and smaller arterioles. The histological findings in my case also indicate that the first changes occur in the glomeruli. The red blood corpuscles, particularly the laked ones, are deposited at first in the glomerular tufts and become arranged at the periphery, giving the impression of becoming adherent to the wall and practically relining it. Only a small central lumen will remain open.

It seems possible that such a relining of the glomerular tufts by red blood corpuscles may greatly interfere with the glomerular filtration and can be the earliest step responsible for the anuria. Finally, the lumen of the glomerular tuft may become blocked completely by the packing of additional corpuscles; thus, hyalin thrombi will arise. This peripheral accumulation mainly of the lighter, laked, crenated, hemolyzed red blood corpuscles and the formation of hyalin tubular structures in the afferent and interlobular arteries appears to be the same phenomenon. The central part of these thrombi with a different structure indicates that the blood stream was probably maintained until the lumen of the afferent arteries was completely plugged by densely packed red blood corpuscles. The structure of the thrombus in the proximal part of the interlobular arteries, presenting irregularly piled up hyalin masses, suggests that there was no central stream left in this part of the thrombus and only packing of the cells occurred. The crevice-like spaces containing a few red blood corpuscles between the arterial walls and thrombi indicate that there was a minimal flow of blood even after the thrombosis was completed.

In the light of the pathological findings observed in the glomeruli one can understand and recognize the possibility of recovery from this disease. If the further deposit of damaged red blood corpuscles should stop, the function of the glomerulus can return. In other words, the glomerular cells, endothelial and epithelial, do not seem to be damaged until thrombosis has obstructed the afferent arteries. Therefore, I believe that the cases reported by

Crook,[7] White[37a] Scriver,[32] Gibberd,[13] and Strumpf[35] can be accepted as probable recovered cases of symmetrical cortical necrosis.

The immediate cause of thrombosis is explained in a number of different ways. Jardine and Teacher,[19] and Cruickshank[9] are in favor of arterial spasm. Oertel and Ash[2] are of the opinion that vasoparalysis and stasis are responsible for the thrombosis, which, however, is relatively terminal. Glynn and Briggs[15] and Von Zalka[38] speak of toxic injury to the endothelium of the kidney, producing thrombosis; Hirst,[18] and Carson and Rockwood[6] are considering an acute toxic glomerular nephritis in association with a toxic injury to the vascular endothelium, while De Navasquez[28] sees the cause in the primary necrosis of the vessel walls.

There is somewhat more unanimity as to the existing relationship between the arterial changes or thrombosis and the cortical necrosis. The great majority of authors maintain that the necrosis is ischemic and due to the thrombosis. The study of the kidneys of my case fully supports the view that the cortical necrosis is secondary to thrombosis. The hemorrhagic border of the necrosis represents exactly the same pathology as the necrotic band and appears an early stage before complete obstruction of the supplying arteries. I was unable to show a primary necrosis of the vessels walls and there was no evidence of inflammatory reaction in the arterial wall. In addition, as in Geipel's[12] case, there were signs of organization of the proximal ends of a few thrombi. Here the arterial wall was in good condition, necrosis of the wall being found only within the necrotic part of the cortex. The finding of organizing thrombi speaks quite decidedly against the view that the thrombosis of the arteries is only a terminal phenomenon.

Although the etiology of renal cortical necrosis is unknown, it seems certain that toxic injury plays the most important rôle. In the cases occurring in pregnant women, some form of toxemia of pregnancy was evident. In the cases of children and men, well recognized infectious dseases became associated with cortical necrosis. This fact suggests the presence of bacterial toxins, at least in a number of instances.

I believe in my case there is a very important point to be emphasized. This is the presence of uterine infection and the finding of very markedly hemoytic streptococci in the thrombus of the right ovarian vein. My interpretation of the rôle played by these organisms is based on the histologic appearance of the red blood corpuscles and on the structure of the arterial thrombi. The uterine infection, judged from the appearance of organized thrombi, was probably present at the time of the spontaneous delivery. Through the many opened uterine veins the hemolytic toxins were rapidly absorbed into the blood stream and hemolysis of the red blood corpuscles occurred.

The finding of a number of distinct hemopoetic foci in the spleen also supports this explanation. The damaged, laked red blood corpuscles became adherent to the endothelial cells of various blood vessels, especially to those of the glomerular tufts. Some thrombi were formed at this time and within a few hours anuria developed. The extensive thrombosis probably required a somewhat longer time. Further absorption of the toxin likely did not occur until late, when the bacterial invasion of the blood stream took place. The venous thrombi are also of recent origin.

Many of the reported cases presented evidence of infection. Such cases were those reported by Juhel-Rénoy,[23] Griffith and Herringham,[16] Rolleston,[31] Cruickshank,[8] Manley and Kliman,[29] Apert and Bach,[1] Davidson and Turner,[10] Kellar and Arnott,[24] zu Jeddeloh,[29] von Zalka,[28] and Evans and Gilbert.[11] Bacteriologic examination was done, however, only in a few cases. For instance, Rolleston obtained pure growth of streptococci from a thrombus in an ovarian vein.

Although there are other hemolytic organisms and certain chemicals, such as dioxan (De Navasquez), which possibly may be etiologic factors, it seems to me that markedly hemolytic streptococci may play a much greater rôle than any other organisms.

Some experiments were carried out by a few investigators who injected hemolytic staphylococcus aureus toxin intravenously into rabbits. Rigdon and his associates found the most conspicuous lesions in the tubules. Thrombosis of the arteries, however, was

not present. Von Glahn and Weld[14] saw fibrinous thrombi in some of the arteries of the kidney cortex and produced necrosis of the nature of infarcts.

It is believed that further experimental investigations are indicated and that a careful bacteriological study of all future cases of cortical necrosis appears to be imperative.

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GLOMERULOMA OF THE KIDNEY*

With Report of a Case and Review of the Literature

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From the standpoint of neoplasia the renal glomerulus has received scant attention in medical literature. Albarran and Imbert,[2] Judd and Donald,[11] Livermore,[13] Hyman,[8, 9, 10] Crawford[4] and Cabot[3] make no mention of the glomerulus of the kidney as a source of tumor formation. Lubarsch[14] says very little on the subject, the only statement being that Wengraf[21] described glomerular-like structures in an embryonal adenosarcoma. Ewing[6] states that in Wilm's embryonal tumors there may be abortive glomerular structures formed by projection of tufts of spindle cells into invaginated tubules.

Weigert[20] found multiple small tumors in the kidneys of a stillborn male infant who had harelip, cleft palate and undescended testes. Both kidneys were slightly enlarged and contained multiple small tumors which were reproducing convoluted tubular structures, glomerular structures and solid masses of cells. His diagnosis was "Adenocarcinoma Renum Congenitum."

Sharkey[16] reported multiple renal tumors, found in a female 28 years of age, which he considered to have arisen from the parietal layer of cells of Bowman's capsule. Her illness began with sharp epigastric pains and pain in the right iliac regions followed by malaise and then by cough. Two months later there were swelling in the hypogastrium, frequency of urination, continuous abdominal pain, emaciation and extreme weakness, enlargement

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of the liver and a tumor mass the size of a cocoanut appeared just above the symphysis pubes. Death occurred one month later, or three months after the onset of symptoms. At the post-mortem examination a generalized carcinomatosis was found involving the lungs, liver, lesser omentum and right ovary. The lower abdominal tumor proved to be an ovarian cyst on the right side which contained no tumor tissue. The ovarian tissue was distributed on the lower and right sides of the cyst and was found to contain tumor tissue. The author, in his description, was uncertain concerning the origin of the tumor but thought that it was from the ovary. On gross examination the kidneys were found to be normal. Microscopically, both exhibited widespread proliferation of the parietal cells of Bowman's capsule and in some areas a proliferation of the lining cells of adjacent convoluted tubules. The lining of these structures, in place of the usual cuboid epithelium, consisted of stratified cuboid and columnar cells exhibiting some variation in size and shape. In some places the groups of cells were quite large. The malpighian tufts were normal throughout. The author's diagnosis of the renal pathology was "carcinoma of the malpighian bodies and tubules of the kidneys."

Hildebrand[7] reported the only case I was able to find that histologically resembled the tumor reported in this paper. His case was a five year old girl operated upon for swelling of the right side of the abdomen. At operation a tumor was found arising from the upper pole of the right side of a horseshoe kidney which was attached caudally. The tumor was encapsulated and the size of a child's head. Several other smaller similar tumors were found near by. The tumor tissue was firm in consistency and was grey, yellow and red in color. Microscopically, it was composed of solid masses of cells with connective tissue trabeculae and areas which the author termed "pseudoglomeruli." These areas were quite variable in size but all of them had fibrous tissue limiting membranes which were lined by a single definite layer of cells. Within these areas there were central groups of cells attached to the lining cells on one side. Hildebrand called the masses "pseudoglomeruli" because there were no capillary tufts

within them. He felt certain that the tumor was the result of an embryonic rest, citing the presence of the horseshoe kidney as evidence of defective development.

Abrams[1] reported a case of a boy 16 years of age who had multiple tumors of the kidneys which he believed to have arisen from the lining parietal cells of Bowman's capsule. The clinical onset consisted of pain in the back followed by emaciation, enlargement of the lymph nodes of the inguinal region, axilla and neck, tumor masses on the surface of the sternum, an enlarged liver, paralysis of the lower extremities, incontinence of the urine and feces and hemoptysis. The postmortem examination revealed a generalized carcinomatosis involving the ribs, sternum, spinal dura, pleura, liver and kidneys. The kidneys were enlarged and contained numerous tumor nodules white in color and firm in consistency. The tumors were formed by a proliferation of the lining cells of Bowman's capsule. The majority of the glomeruli had normal vascular tufts, although in a few instances the proliferation of the parietal cells filled the entire capsule. In some areas the proliferation destroyed and penetrated the basement membrane of the capsule and infiltrated the surrounding tissue. Most of the tubules were normal, although some of them were invaded and replaced by tumor tissue. The cells of the tumor tissue closely resembled the normal lining epithelium of the capsule. Elsewhere in the body the tumor tissue was composed of cells which were slender and packed in irregular alveoli, the centers of which exhibited considerable necrosis. He cited the case of Sharkey[16] and felt that the renal tumors in his case were identical with those in Sharkey's case. He did not state whether he thought the renal tumors were related to the generalized carcinomatosis, or whether he thought that they were incidental.

Weber[18,19] reported two cases in both of which he described structures which he termed "a giant neoplastic imitation of the malpighian tuft." In his second report[19] he used the term "Glomerular nephroma." His first case was that of a male aged 66 years who died as a result of an injury. Both kidneys were granular to a slight extent. The right kidney contained a par-

tially necrosed Grawitz tumor; the left contained many minute white nodules in the cortex which were just beneath the capsule and which looked like tubercles. Microscopically, he found them to be neoplastic and from their morphology termed them "giant neoplastic imitations of malpighian tufts." His other case was that of a 63 year old male who died as a result of heart disease. His kidneys were large and granular, and contained multiple nodules which varied in size up to the size of a pea. Some of them were white in color and some were brown. One of the nodules proved to be a leiomyolipoma and some were papillary adenomata which were intracystic and represented attempts to form malpighian tufts in a "rude neoplastic giant sort of way." Weber stated that similar structures were found by Kelynack[12] but after reviewing Kelynack's report I am unable to agree with Weber and do not believe that the structures described by Weber and Kelynack are similar. Weber pointed out that Sabourin[15] had previously called attention to the connection between papillary adenomata and chronic renal fibrosis and felt that there must be some relationship between senile changes and chronic irritation or infection on the development of tumors. He cited cirrhosa adenomatosa of the liver as an example of chronic fibrosis exciting new growth formation.

Turley and Steel[17] observed, in an adult 43 years of age, multiple small miliary adenomata of the kidney which they believed to have arisen from, and to be reproducing, glomerular neoplastic structures. In their case the renal pathology was incidental to and not related to the cause of death, although the cause of death was not stated. Both kidneys were small and contained multiple small cortical tumors varying in size from that of a pin head to that of a pea. The tumors were composed of branching and anastomosing capillaries covered by epithelial cells which were cuboidal and cylindrical in shape and arranged in single and double layers along the capillaries. A few of the cells contained mitotic figures.

Dschu-Yu-Bi[5] reported a case in which there were multiple unilateral renal tumors, some of which were glomerular in structure. The specimen which he described was removed surgically

from a male age 37 years because of pain in the lumbar region and hematuria. The kidney was enlarged, measured 18 x 18 x 7 cm. in size, and contained multiple tumors of various sizes and types. Some of them were adenomata with lipoid containing cells; others were what he termed "intracystic papillomata" which were glomerular in architecture; still others were adenomata which resembled convoluted tubules. In those which resembled glomeruli there were no capillary tufts.

Thus in my search of the literature (see table of cases) I have been able to find eight previously reported cases which can be

TABLE 1

GLOMERULOMA OF KIDNEY. TABLE OF CASES

AUTHOR	YEAR	AGE	SEX	SYMPTOMS	DIAGNOSIS AT	NUMBER OF TUMORS	KIDNEY SIZE	MICRO
Weigert	1876	NB	M	No	PM	Multiple	Slightly enlarged	M
Sharkey	1882	28	F	Yes	PM	Multiple	Normal	M
Hildebrand	1894	5	F	Yes	Op	One large, few small	Enlarged	M
Abrams	1899	16	M	Yes	PM	Multiple	Enlarged	M
Weber	1917	66	M	No	PM	Multiple	NA	B
Turley and Steel	1924	43	NA	No	PM	Multiple	Small	M
Weber	1925	63	M	No	PM	Multiple	Enlarged	B
Dschu-Yu-Bi	1934	37	M	Yes	Op	Multiple	Enlarged	M
Owen	1937	52	F	Yes	Op	Solitary	Normal	M

NA: Not available; PM: Post mortem; Op: Operation; M: Malignant; B: Benign.

classified as glomeruloma of the kidney. Two of the cases, (Sharkey and Abrams), consisted of neoplastic proliferation of the lining cells of Bowman's capsule without reproduction of and with little involvement of the malpighian tufts. Seven of the cases were multiple small tumors. Only one (Hildebrand's case) was a large solitary tumor and it contained a number of smaller tumors. Six of the eight tumors were considered as malignant, including the large solitary tumor of Hildebrand. Six of the cases were discovered at postmortem examination, two of them at operation. Of the two cases discovered at operation, no

follow-up is available, although in each instance the kidney was removed and the patient recovered from the operation.

REPORT OF CASE

Mrs. J. J., 52 years of age, was admitted to the Grace Hospital, Detroit, in October 1931, complaining of pain in the right lumbar region and frequency of urination.

She had two children, aged 31 and 27 years, living and well. Her husband was living and well. One of her grandfathers had a carcinoma of the lip. Her mother died at 73 with uremia. Her father died at 68 following an operation for hernia.

Twenty-seven years previously, following the birth of her youngest child, she was in bed for four weeks with a puerperal infection following which she made a complete recovery. In April 1921 she had a radical removal of the right breast for scirrhous carcinoma. While convalescing from this operation she had an attack of right-sided pyelitis from which she made a prompt recovery. Following the mastectomy a sinus persisted in the axilla. In October 1921 she was studied thoroughly and no metastases were found in the right shoulder region by roentgenological examination or anywhere else in the body by physical examination. Urine and blood studies were normal and a Wassermann test was negative. Radium was then applied over the sinus but it failed to heal. In January 1922 the tissues about the sinus were resected and postoperative radiation was applied. Healing was prompt and no further recurrence took place. Scirrhous carcinoma was found in the resected tissues. From that time on she was subject to periodic attacks of pain in the lumbar region and frequency of urination, the attacks coming on at irregular intervals.

In 1928 she had an unusually severe attack of pain in the right lumbar region accompanied by frequency of urination. At this time she was thoroughly examined for evidences of metastases from the breast cancer of seven years previous to that time but none could be found by physical or roentgenological means. Blood chemistry studies yielded normal results. The urine from each kidney obtained by ureteral catheterization was chemically and microscopically normal. Pyelograms of each kidney were normal. Septic tonsils were found and were removed, without beneficial results, however, as far as the pain in her right lumbar region was concerned.

Following the 1928 episode the pain in the lumbar region was more or less constant and described by the patient as dull and aching. It did not radiate at any time. The frequency of urination continued but the relationship to the pain in the lumbar region became less evident.

The physical examination in October 1931 was entirely negative, except for the mastectomy scar and a moderate amount of tenderness which could be elicited on bimanual palpation of the right kidney area, the kidney itself not being palpable.

On cystoscopic examination the urethra and bladder were found to be normal. Catheters passed easily into both ureters and no obstructions were met. Phenolsulphonephthalein injected intravenously made a normal appearance time and was excreted normally. Under fluoroscopic observation sodium

FIG. 1. Unretouched photomicrograph. Magnification 110 diameters. The upper border is the margin of the central area of necrosis. Three cholesterin clefts are to be seen. Between the zone of necrosis and the tumor tissue there is a layer of dense fibrous tissue. The lower one-half is tumor tissue which has a slight trabecular connective tissue framework.

iodide was injected into the pelvis of each kidney. The left side was found to be normal. The right pelvis was slightly dilated and appeared less mobile than usual. On close observance dribbles of fluid could be seen going down the right ureter with no relation to pelvic contractions and with no particular rhythm. The right kidney appeared to be fixed at the lower pole and on

inspiration descended and rotated in an arc with the pivot at the lower medial aspect. When the ureter filled with the iodide no obstructions or kinks were observed. Urine samples obtained by catheterizing the bladder and ureters separately were found to be normal by chemical and microscopic examination and yielded no organisms by smear or culture.

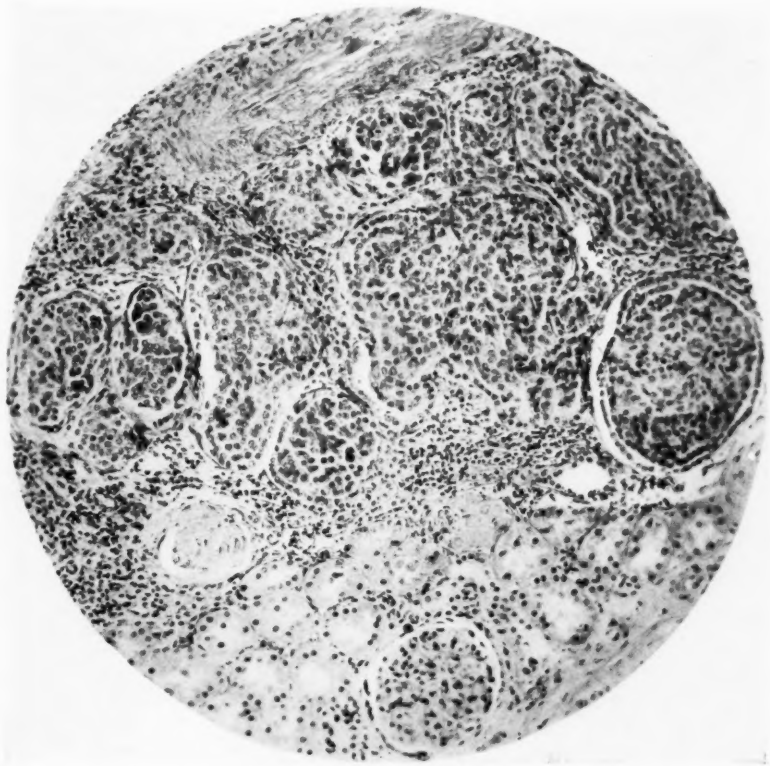

FIG. 2. Unretouched photomicrograph. Magnification 110 diameters. A normal renal glomerulus surrounded by normal tubules is at the lower edge. Observe the glomerular masses of tumor tissue surrounded by a single layer of cuboid cells. The stroma in this picture exhibits some lymphocytic infiltration.

She was operated upon November 12, 1931. Under general anesthesia the right kidney was removed through the usual Mayo incision. During the removal it was found to be densely adherent at the medial aspect of the lower pole, which area corresponded to the area of fixation seen by fluoroscopic examination while the pyelogram was being done. Recovery from operation

was uneventful. There was no postoperative radium or roentgen therapy. At the present writing (May, 1937) the patient is well and has no symptoms referable to the renal lesion.

On gross examination the kidney was not enlarged. It contained a solitary tumor in the lower pole at the medial aspect. The tumor was spherical in shape, two and one-half centimeters in size and presented at the surface.

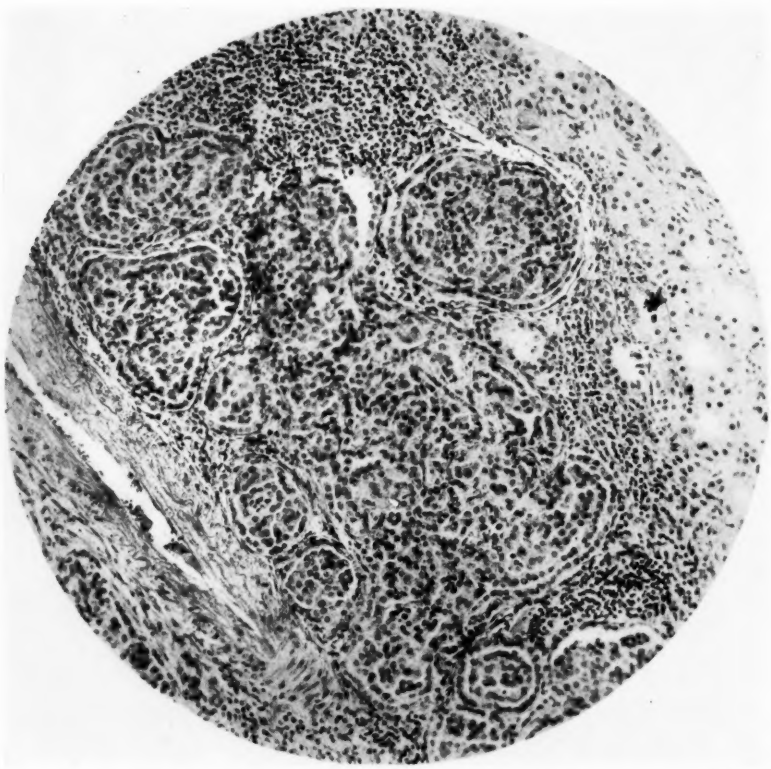

FIG. 3. Unretouched photomicrograph. Magnification 110 diameters. A few normal tubules are present at the upper right border. There are a large number of lymphocytes at the periphery of the tumor tissue. Observe the glomerular architecture and the capsule which surrounds the cell masses.

When incised it was found to be quite hard in consistency except in the center, where there was a small amount of white pultaceous material. It was white in color with some yellow mottling. Except for the tumor the kidney was grossly normal.

On microscopic examination the kidney tissue proper is found to be normal

except for a few scarred glomeruli and a few tubules containing hyalin casts. These changes are found only in the areas adjacent to the tumor.

The stroma of the tumor is quite variable. In the center about the pultaceous material it is quite dense and abundant.

It is likewise quite dense about the periphery, especially at the surface but adjacent to the renal tissue in some places. In other areas adjacent to the

FIG. 4. Unretouched photomicrograph. Magnification 565 diameters. This is a picture of one of the glomerular masses. Observe the tuft of cells attached to one side and the peripheral layer of lining cells which are surrounded by connective tissue.

renal tissue it is entirely absent, thus failing to form a capsule. Elsewhere throughout the tumor it is composed of narrow connective tissue trabeculae which separate the growth into lobular masses. In some places, and especially about the periphery, there is a rather pronounced lymphocytic infiltration. The central area of pultaceous material is surrounded by a zone of semi-necrosis

which contains a small amount of deep yellow crystals, a few phagocytic mononuclear cells and a few cholesterin clefts (fig. 1). The parenchyma of the tumor consists, at the periphery, of well demarcated glomerular structures without any vascular tufts (figs. 2 and 3). Most of them are well separated from the adjacent tissue by fine fibrous tissue trabeculae. The glomerular structures have a peripheral limiting membrane which is lined by a single layer of cuboid cells which exhibit some piling up and some anaplasia. The glomerular structures consist of masses of cells similar to the lining of the peripheral membrane and are attached to the peripheral cells at one or more points. These masses of cells (fig. 4) are somewhat anaplastic varying some in size, shape and staining qualities. The cytoplasm is non-granular and has indistinct borders; the nuclei are round, some of them are hyperchromatic and an occasional mitotic figure is present. The glomerular units are quite variable in size and shape but most of them are round and larger than normal glomeruli; a few are smaller and a few are about the same size as normal glomeruli. The smaller ones are seen at the periphery, whereas in the center they are much larger and lose somewhat their glomerular architecture, always retaining, however, their separation by fine connective tissue trabeculae. All of the trabeculae are continuous and many of them, regardless of the size of the encircled mass, continue to have a lining of a single layer of cells to separate them from the masses of enclosed cells. None of the glomerular masses contain capillary tufts or blood vessels.

DISCUSSION

Since a malignant tumor of the breast had been removed ten years previously, the question naturally arises whether there is any relationship between the two tumors. As there were no avabilable sections of the original mastectomy comparison could not be made. However, through the courtesy of Dr. Curtis Burnam of the Howard A. Kelly hospital, sections were available from the recurrence which was removed from the axilla about the sinus which remained following the original operation and no similarity could be made out between the kidney tumor and the recurrent carcinoma.

From an embryological standpoint the origin and histogenesis of this tumor can be readily explained. During the differentiation of the permanent kidney from the nephrogenic tissue, metanephric spheres are formed first, these in turn being converted into vesicles which have eccentrically placed lumina. These vesicles, after a sequence of proliferation and an ingrowth of the capillary tufts, are converted into the nephrons or secreting

units of the kidney which comprise the malpighian body, the proximal and distal convoluted tubules and the loop of Henle. This tumor, then, arises from the metanephric vesicle before it differentiates into its ultimate components and before the glomerular portion is penetrated by a capillary tuft. It could be termed a "metanephroma" since only metanephric tissue participates in its formation. The term "glomeruloma" is preferred, however, because of its architecture. Its presence in a woman 52 years of age can most easily be explained on the basis of an embryonic rest which has either been growing slowly for many years, or which has been quiescent for many years and recently become activated. Microscopically, it is a malignant tumor because of its invasive properties and because of the anaplasia of the cells. Clinically, however, it has behaved as a benign tumor since it has not recurred after an interval of five and one-half years following removal.

There are several points of interest in this case study. The patient is now cured for 16 years of a carcinoma of the breast which recurred after removal. She is cured for five and one-half years of a glomeruloma of the kidney. The initial symptom which may have been caused by the glomeruloma of the kidney occurred, possibly, more than ten years before its removal. The renal tumor was not diagnosed preoperatively, but, by means of the fluoroscope and a pyelogram a perirenal adhesion was diagnosed, thus leading to the operation upon the kidney.

CONCLUSION

By review of the medical literature, by a consideration of the embryology concerned and by the report of a case, the entity of "Glomeruloma" of the kidney is proposed.

The author wishes to express his appreciation to Dr. H. W. Plaggemyer, Urologist-in-chief of the Grace Hospital, and to Dr. Carl Weltman for supplying the clinical records in this case and for permission to use the case herein reported.

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ENDOMETRIAL HISTOLOGY IN RELATION TO OVARIAN FUNCTION*

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The ovary, unlike most endocrine glands, has a mirror in which it reflects its activity. This mirror of ovarian activity is the endometrium. In many respects study of this functional reflector of the ovary has been extremely valuable to those interested in the clinical phenomena associated with normal as well as well as with abnormal ovarian function. On the other hand, this same tissue has been the source of much confusion in the literature and of many questionable conjectures regarding the interpretation of ovarian function as manifested by the histologic changes observed in the endometrium.

Following study of several hundred specimens of endometrium removed from the uterus at dilatation and curettage or for biopsy or after hysterectomy,[1,3,4] I previously proposed a newer classification of endometrial changes, based on descriptive histologic terms which denote at the same time the functional activity of the ovarian hormones controlling these changes. This classification of the normal cycle is briefly restated here and will serve as the nucleus around which discussion of abnormal ovarian function will center.

CLASSIFICATION OF NORMAL CYCLE

Menstruating phase. Several specimens were obtained during the phase of "tissue loss" and, according to the observations made, there can be no doubt that loss of tissue is complete in the first twenty-four hours of menstruation and that the remainder of the period is one of hemorrhage and secretion. This

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observation is in accord with observations made by Novak and others. Some observers, however, do not concur in this view. Figure 1 illustrates this tissue loss. The specimen was obtained twenty-four hours after onset of the menstrual period of a patient whose cycle may be considered normal. At this time tissue loss amounts to approximately three-fourths of the endometrium; there remains only the basal layer of endometrium, which measures approximately 0.5 mm.; this picture might well be confused with that of freshly curetted uterus. The glands in the basal layer remain essentially the same throughout the cycle;

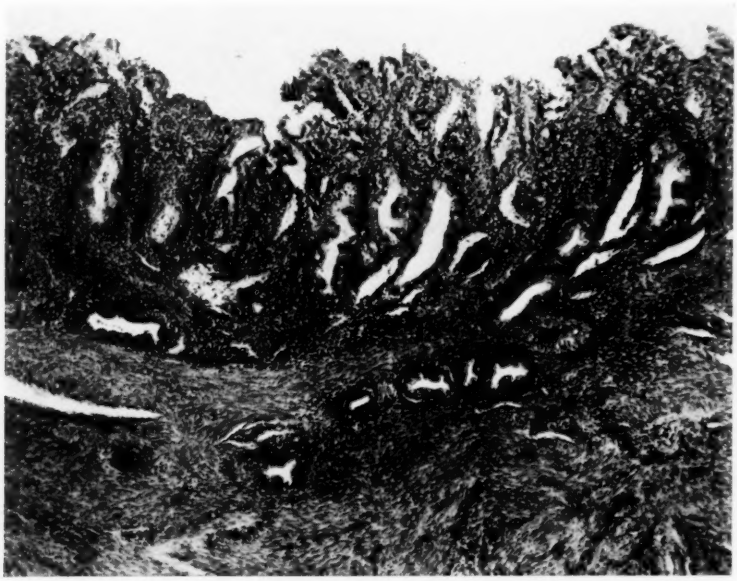

FIG. 1. MENSTRUATING PHASE ("TISSUE LOSS")

Specimen obtained 24 hours after onset of menses in a normal cycle. Subbasal glands and loss of outer two layers of endometrium. Hematoxylin and eosin, ×50. (From Herrell and Broders: Surg., Gynec. and Obst.)

hence, very little can be learned concerning the phase from such specimens, when obtained either by dilatation and curettage or from the whole uterus. The surface and outer layer of endometrium, however, afford means of determining accurately the phase of the cycle, although this is as easy to determine from specimens removed at dilatation and curettage as in a block from the whole uterus, assuming the sections are prepared properly. The small glands which will be seen below the basal layer of endometrium, should be noted. Of particular significance also is the height of the epithelium and the general configuration of these glands. The term "sub-basal" has been proposed for these glands and reference to them will be made in a later paper.

Early repair phase of cellular migration and rearrangement. In the next twenty-four hours appears the phase of reorganization, rearrangement, and migration of cells. From microscopic study it is possible to learn that the cells lining the remaining glands will take part in resurfacing of the endometrium.

Early proliferative phase (first to seventh day). This phase corresponds roughly to that which previously, and I believe incorrectly, has been called the "post-menstrual phase." It is characterized by active cellular division, resurfacing of the endometrium and formation of new, straight tubular glands

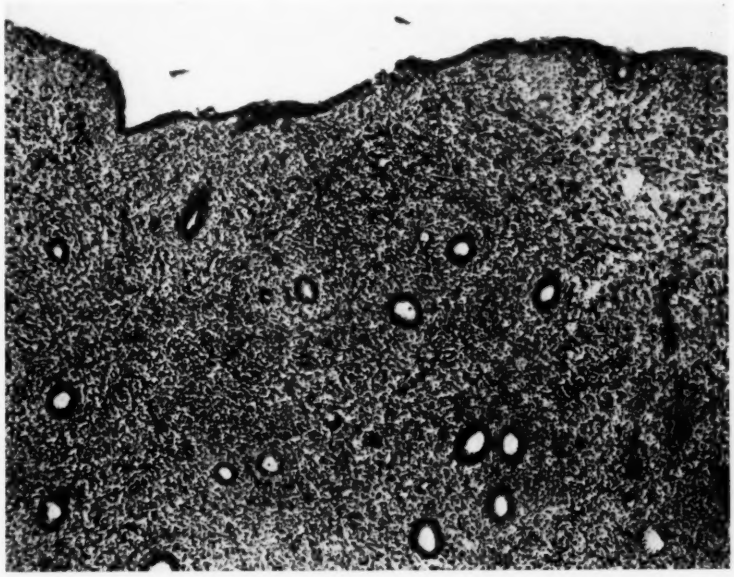

FIG. 2. EARLY PROLIFERATIVE PHASE (CROSS SECTION)

Specimen obtained on fifth day of cycle, illustrating early proliferative phase when glands are seen on cross section only. Other features similar to early phase. Hematoxylin and eosin, ×50. (From Herrell and Broders: Surg., Gynec. and Obst.)

from the surface epithelium. Mitosis is active and cellular proliferation occurs also in the loose embryonal type of stroma. By the end of the first week in this phase the endometrium presents a fairly typical picture. The average number of glands per low power field is three to four and these glands are nearly straight tubules, the epithelium of which is moderately low columnar in type, with nuclei situated near the centers of the cells. The endometrium grossly is from 1 to 1 + mm. thick. Therefore, it is possible at once to state the time of the last period and hence the phase of the new cycle. These glands always should be studied, if possible, in the longitudinal view; if they are seen on cross section, however, the phase is discernible by the characteristics just outlined.

The tubular glands will appear in cross section merely as small circles with other features of the early proliferative phase (fig. 2).

Late proliferative phase (eighth to fourteenth day). This phase (fig. 3) corresponds roughly to what previously has been called "interval" or "resting endometrium" and it is with this phase that I especially wish to deal. The regenerative process here is not that of a resting endometrium nor is there a resting period. The very nature of the process of cyclic regeneration prevents such an occurrence. During this late proliferative phase, proliferation con-

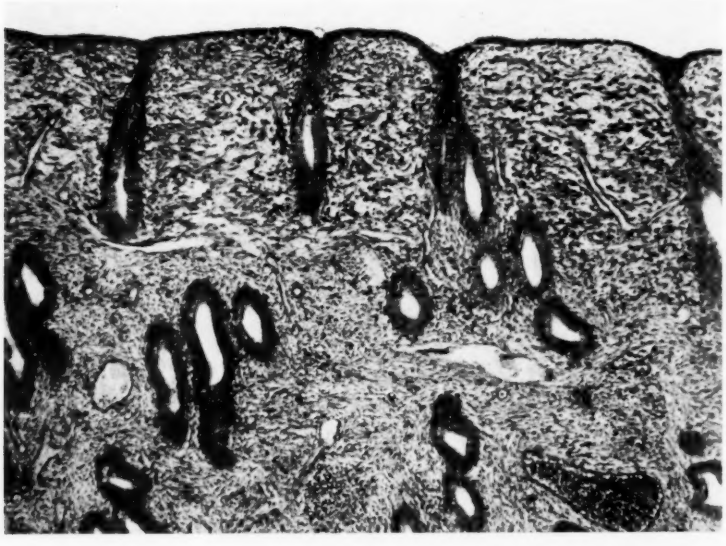

FIG. 3. LATE PROLIFERATIVE PHASE

Specimen obtained from a patient with a normal cycle, on the ninth day. Straight tubular glands somewhat dilated as compared with early phase. They average 6 per low power field. Gross thickness of endometrium, 2 mm. Hematoxylin and eosin, ×50.

tinues rapidly; new glands form from the surface epithelium and the stromal cells in a given field nearly double in number. By the end of the second week of this phase, the average number of glands per low power field is six to seven; these glands are somewhat dilated but they remain of the straight, tubular type and show little, if any, evidence of a differentiative change. The endometrium grossly is from 2 to 2.5 mm. thick. It is worthy of note that this phase corresponds roughly to the life of the follicle and therefore it would seem that the follicle has to do with the proliferative phase of this process of regeneration. At the end of this phase, proliferation is on the decline; however, evidence of

slight proliferation is seen in the next phase, another striking correspondence with the failing follicular activity.

Early differentiative phase (fifteenth to twenty-first day). This also has been called the "interval phase" by Novak; by others it has been called the "phase of premenstrual endometrium." I would again propose descriptive terms which would at once indicate the histologic as well as the physiologic state of the endometrium. One of the earliest indications of differentiation is the beginning convolution of the longitudinal glands. The epithelium, which up

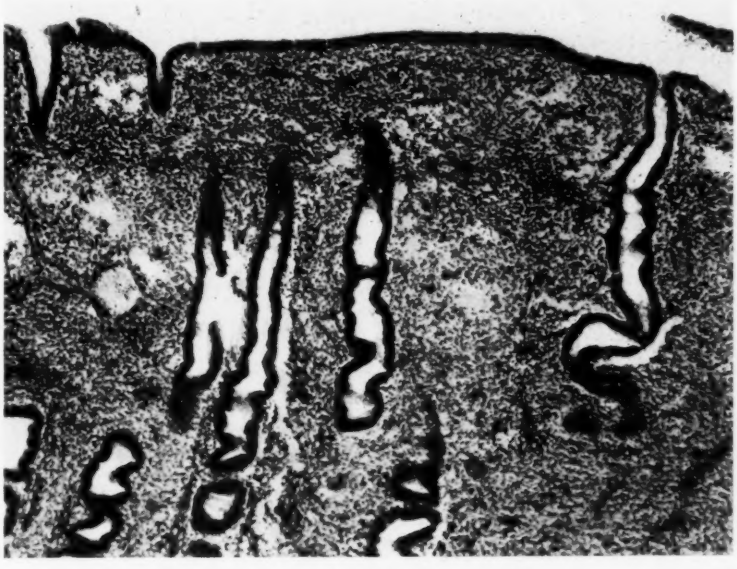

FIG. 4. EARLY DIFFERENTIATIVE PHASE

Specimen obtained for biopsy from patient with normal cycle, on the fifteenth day. Beginning convolution of longitudinal glands. Six to seven glands per low power field. Gross thickness of endometrium 2.5 cm. Hematoxylin and eosin, ×50. (From Herrell and Broders: Surg., Gynec. and Obst.)

to this time has been of the proliferative type, that is, low columnar epithelium, changes to the columnar type and the nuclei approach the bases of the cells. There is some evidence of proliferation, namely, an increase of cells in the stroma. By the end of the third week of this early differentiative phase the endometrium grossly is about 3 to 3.5 mm. thick; the number of glands, however, is not increased as compared with the proliferative phase, the average number remaining six to seven per low power field (fig. 4). These glands are dilated and there is only an apparent increase in the stromal cells, which has been called by most workers "continued proliferation." It is of note that

this phase is identical with that of early activity of the corpus luteum and failing follicle, which histologically is expressed here as decreasing evidence of proliferation and a rather sudden onset of all of the histologic evidence of differentiation. It is with the early part of this phase that one may have difficulty in classifying endometrium, especially in fresh section. The fixed preparation, however, using the criteria outlined, will supply the necessary information.

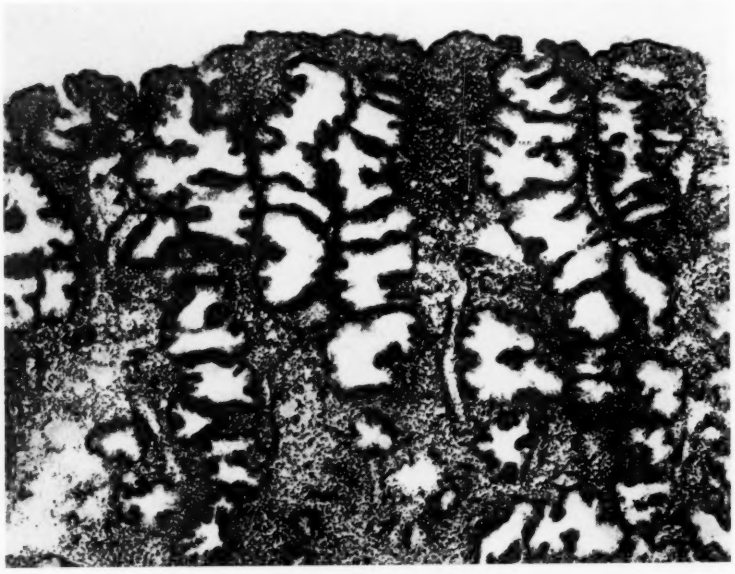

FIG. 5. LATE DIFFERENTIATIVE PHASE

Specimen obtained for biopsy on twenty-fifth day from a patient whose cycle was entirely normal. Typical late differentiative phase, that is, six to seven glands per low power field, glands twisted on longitudinal axes, and tall columnar epithelium. Gross thickness of endometrium, 4 mm. Hematoxylin and eosin, ×50. (From Randall and Herrell: Surg., Gynec. and Obst.)

Late differentiative phase (twenty-second to twenty-eighth day). This may be termed the "premenstrual phase," although such a term does not describe the true state of endometrium. In this phase differentiation is at its height and is characterized by certain changes. The glands which in the early differentiative phase disclosed beginning convolution, are now twisted on their longitudinal axes, producing the typical corkscrew glands or, to use Schröder's term, "sageförmigedrüsen." When these glands are viewed in longitudinal section they look like a sectioned seashell with the typical saw-tooth appearance of the

epithelium. This twisting, I believe, is a characteristic of differentiation whereby the surface area is increased. The epithelium is distinctly columnar and the functional activity is marked. There is a definite cytoplasmic increase, with a decreased nucleocytoplasmic ratio, a distinct differentiative phenomenon. Glycogen material is increased in these tall cells and the nuclei are displaced nearly to the basement membrane. The lumens of the glands at this phase are increased in size, and hence the stroma, by mere mechanical pressure, apparently is increased. By the end of this phase the endometrium grossly is approximately 4 mm. thick, yet the average number of glands per low power field remains the same as at the end of the proliferative phase (fig. 5).

Just prior to menses there is premenstrual engorgement of the stroma and infiltration with wandering cells, together with some edema near the surface. This engorgement is a generalized vascular reaction in the arterioles, in definite contradistinction to the focal hemorrhagic areas seen in specimens obtained at dilatation and curettage.

CLASSIFICATION OF OVARIAN DYSFUNCTION

We have adopted, at The Mayo Clinic, a fairly simple clinical classification of the various types of ovarian dysfunction. In general, they are divided into two large groups: the "primary ovarian dysfunctions" and the "secondary ovarian dysfunctions." In the former group failure is primary in the ovary itself. In this group, one is confronted not only with the problem of menstrual irregularity, and often sterility, but also with a train of symptoms associated with true ovarian failure. There is no evidence in this group of pituitary disturbance such as abnormal fat metabolism, abnormal water metabolism or lowered basal metabolic rate.

The second large group includes the primary pituitary and thyroid failures in which ovarian failure is, of course, a secondary phenomenon. In this group evidence of disturbances of fat metabolism as well as of water metabolism and lowered basal metabolic rate often are found. This, roughly of course, constitutes the clinical classification.

From the histologic standpoint the evidence of ovarian dysfunction, however, is the same in both groups since the endometrial pattern changes with ovarian function regardless of whether the cause of its abnormal function comes from within or from without the ovary. It becomes apparent, therefore, that

one cannot classify these dysfunctions on the basis of the histology of the endometrium only, but histologic examination does form a part of the armamentarium in arriving at a rational diagnosis. Biopsy of the endometrium, with study of its histologic characteristics, is of greatest value in recognizing a subdivision of these ovarian dysfunctions into the groups of partial or complete ovarian failure, regardless of whether the failure is primary or secondary. Since the regenerative phenomenon seen in the endometrium depends on the ovarian hormones for its stimulus, any degree of failure of this stimulus, or stimuli, can be recognized. The proliferative phases of the endometrial cycle, as has been seen, are dependent on the follicular hormone, and the differentiative phases depend on the normal activity of the luteal hormone. If either of the hormones is absent or deficient, then the regenerative phases likewise will be absent or deficient. Further, the prolonged activity of one or the other hormone will result in prolongation of the phase controlled by this hormone.

On this basis of this histologic and physiologic explanation I have proposed the term "persistent phases of the cycle" which at once denotes the deficiency in ovarian function which is manifested by the endometrial change. The interpretations have been consistent to the degree that often it is possible to predict the type of clinical syndrome associated with a given histologic pattern.

As examples of the persistent phases mentioned above, which are associated with ovarian dysfunction, a few of the representative tissues are here presented. Before proceeding, however, it is pertinent to point out again, very definitely, that one must not depend alone on histologic examination in these cases, but it is extremely valuable to combine, intelligently of course, knowledge of the histologic picture with knowledge of the clinical picture and of results of other laboratory examinations in making diagnosis of these dysfunctions. Additional laboratory aids include study of the patient from the point of view of determining possible pituitary dysfunction, basal metabolic rates, physical examination, and estimation of urinary estrin and prolan when possible. These laboratory procedures, however, as indicated before,

make possible clinical classification of the primary and secondary ovarian failure groups while biopsy and endometrial study in itself is the invaluable and almost the greatest single diagnostic aid in determining the degree as well as the kind of ovarian dysfunction, either partial or complete.

It is essential to obtain for biopsy a representative specimen of endometrium and this can be accomplished, as a rule, without difficulty in the office. The Randall[4] biopsy instrument offers the most satisfactory way of obtaining these tissues. In fact, the specimens so obtained are as satisfactory as those obtained by dilatation and curettage. One important feature must be remembered, however. *To obtain the desired information in the ovarian failure group, the specimen always should be taken between the eighteenth and the twenty-fifth days of the cycle, regardless of whether the cycle is considered normal or abnormal, that is, the specimen always should be obtained between the eighteenth and twenty-fifth days after the onset of the last period of bleeding.* The reason for this is obvious, namely, the fact of follicular failure or failure of the corpus luteum can be learned only after the period of normal activity of these hormones has passed. If, for example, a specimen for biopsy is taken on the tenth to the twelfth day and the proliferative effect is seen, this is of course of normal finding and ovarian failure may be overlooked. However, if evidence of a proliferative phase is found on the twentieth day, which is the time when differentiation should have been well established, it is clear at once that deficiency of corpus luteum exists. This type of endometrium is said to be in the "persistent proliferative phase" and the stimulus to differentiation (corpus luteum) is lacking. What has been described will prove true not only from a physiologic standpoint but from a clinical standpoint.

Now it is proper to proceed with data on the classification of persistent phases of the cycle.

Persistent early proliferative phase. The word "persistent" used in this classification denotes the histologic state of the endometrium and at the same time postulates abnormal function of the ovary. For example, an ovary deficient in production of luteal hormone and to some degree deficient in follicular produc-

tion will express these changes by a lack of development (or regeneration) of the endometrium, and the endometrium, therefore, will remain in a state of persistent proliferation. Such an endometrium is not hypertrophied or hyperplastic in any sense of the word—in fact, often such an endometrium will appear microscopically the same as the endometrium usually seen in the first

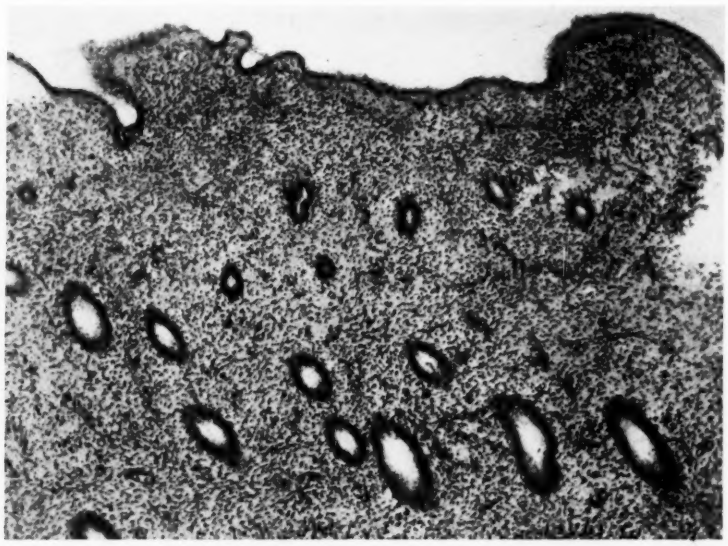

FIG. 6. PERSISTENT EARLY PROLIFERATIVE PHASE OF MENSTRUAL CYCLE

Glands identical with those shown in figure 2; they are small, straight tubular glands lined with low epithelium. This endometrium does not differ from normal endometrium in any respect except that it was removed in the last part of a cycle. No actual increase in the total number of glandular elements (although previously called hypertrophied or hyperplastic endometrium). Average thickness of endometrium, 1 mm. Hematoxylin and eosin, ×50.

seven days of the normal cycle. It is obvious, therefore, that a pathologic diagnosis need not be made but rather a report of the degree of ovarian failure can be given. In addition to histologic accuracy, such a report is of more diagnostic value to the clinician than is a pathologic diagnosis. The specimen shown (fig. 6) represents an endometrium removed on the twentieth day of an abnormal cycle. This specimen, if it had been taken on the

twentieth day from a patient whose cycle was normal, should give evidence of differentiation, which is a normal corpus luteum effect. However, histologic study discloses that the endometrium is arrested in the proliferative phase. The patient was a bleeder who previously had been given theelin; incidentally this produced more, rather than less, bleeding. It becomes clear that this patient did not lack theelin, which is the proliferator of the endometrium, but rather the deficiency is one of stimulation by corpus luteum. The treatment indicated, therefore, is one which is designed to bring about stimulation of the corpus luteum. The patient under consideration likewise was given proluton and antuitrin S, and the menorrhagia ceased. This same type of endometrium may also be seen occasionally in cases of amenorrhea; nevertheless, the basic disturbance in the ovary is the same. The problem of the exact cause of bleeding obviously still baffles students of this subject.

In addition to using the available hormones on the market as means of ovarian stimulation, at the clinic we have been successful in treating patients who have amenorrhea by means of roentgenologic stimulation over the ovary, and when indicated, over the pituiary gland.* There are several advantages in this type of therapy, the most important of which is the earlier response to treatment; another advantage is that monthly injection of the glandular products is not necessary. Financial and geographic difficulties both are met by means of roentgen therapy without additional burden to the patient. The dosage of roentgen rays which we are using has been stated recently in a publication by Dr. Erips.

Persistent late proliferative phase. The principles outlined in the preceding paragraphs apply in examination of specimens in this phase. Again the endometrium reflects the state of ovarian

* For the treatment of ovarian dysfunction, one field is irradiated over each temporal region, centering over the pituitary, and one field over the front of the abdomen, centering over each ovary. The following technic is used: 200 kilovolts, 10 milliampers, 0.75 mm. copper and 1 mm. aluminum filters, 50 cm. distance for five minutes. This represents approximately one-sixth of a skin erythema dose, and may be repeated if not effective in three months.

function and the difference herein is one of degree rather than of kind. A persistent late proliferative phase found in examination of a specimen of endometrium removed in the last part of a regular or of an irregular cycle denotes merely that a full proliferative or follicular effect has been exerted on the regenerating endometrium. The only ovarian deficiency evident in this phase, therefore, is in production of the luteal hormone. Again, the

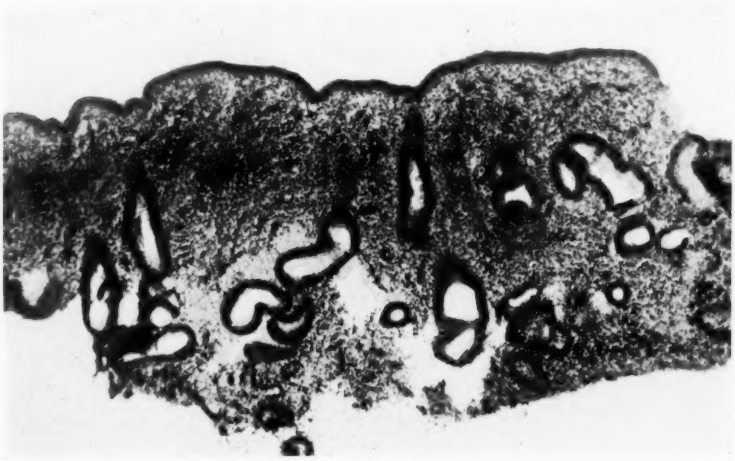

FIG. 7. PERSISTENT LATE PROLIFERATIVE PHASE OF THE MENSTRUAL CYCLE

Average thickness of endometrium approximately 2 mm. Glands nearly identical with those found in same phase of cycle in a normal endometrium (fig. 3) in the late proliferative phase. Average number of longitudinal glands per low power field is six. Specimen was removed from a patient with menorrhagia. Hematoxylin and eosin, ×50. (From Counseller and Herrell: Jour. Indiana State Med. Assn.)

change is not hyperplasia but arrest of the regenerative phenomenon. Patients whose tissues can be classified in this group, interestingly enough include the largest number of bleeders and, therefore, constitute clinically one of the most important groups of patients. The treatment likewise is not by administration of theelin but of a luteinizing hormone. The endometrium usually will have characteristics almost identical with those of a normal late proliferative phase of a normal cycle. The glands

are somewhat dilated but they are straight, tubular glands; approximately six such glands are found per low power field. The gross thickness of the endometrium usually is not more than 2+ mm. Figure 7 represents a state of persistent late proliferation in an endometrium removed on the twenty-third day of the cycle. In addition to menorrhagia, the patient also had had repeated miscarriages. Following three months' treatment with

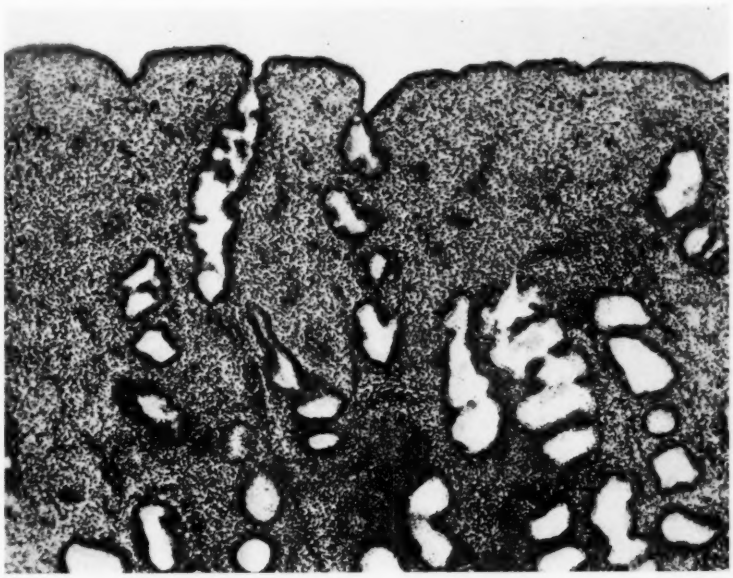

FIG. 8. LATE DIFFERENTIATIVE PHASE OF THE MENSTRUAL CYCLE

Specimen removed from endometrium of the same patient whose endometrium is shown in figure 7. This endometrium is entirely normal and was obtained after five months' treatment. Hematoxylin and eosin, ×50. (From Counseller and Herrell: Jour. Indiana State Med. Assn.)

a luteinizing hormone, the next specimen was obtained (fig. 8). This specimen is of typical differentiative endometrium which can result only from stimulation by corpus luteum. Clinically the patient recovered from the menorrhagia; also she became pregnant and was delivered normally of a living child.

Persistent early differentiative phase. The types of abnormal ovarian function associated with tissue classified in this group

are interesting. A state of persistent early differentiation denotes, as would be expected, partial failure of corpus luteum and again the endometrium appears like that seen in the third week of the normal cycle, in which there has been a partial but not a complete luteal effect on the endometrium. Bleeding is less

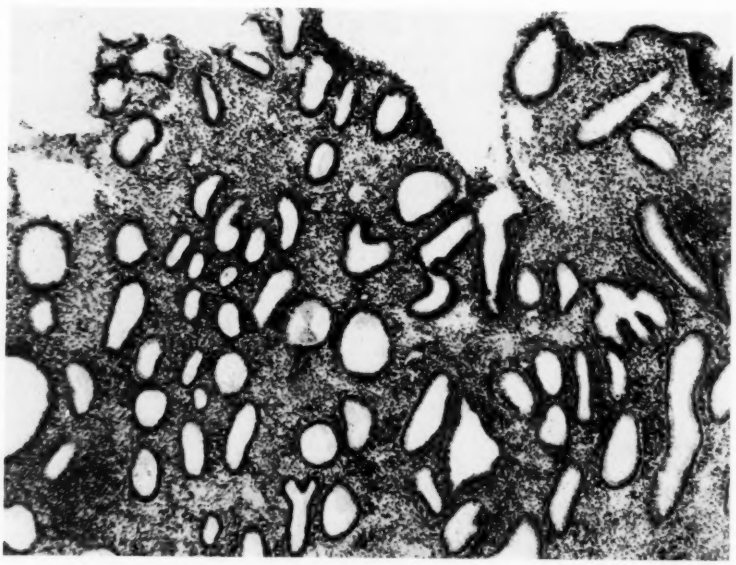

FIG. 9. PERSISTENT EARLY DIFFERENTIATIVE PHASE OF THE MENSTRUAL CYCLE IN A CYSTIC ENDOMETRIUM

Longitudinal glands characteristic of the early differentiative phase. Glands lined by columnar epithelium and have convolutions which are normal (fig. 6) for the early differentiative phase, that is, the fifteenth to twenty-first day of the cycle. Average number of longitudinal glands per low power field is six to seven. Endometrium differs from the normal only by the presence of cystic areas lined by flat, nonfunctioning epithelium. Some effect of the corpus luteum hormone evident in the endometrium. However, absence of complete differentiation in presence of cysts indicates of partial failure. Hematoxylin and eosin, ×50. (From Randall and Herrell: Surg., Gynec. and Obst.)

among the patients corresponding with endometrium of this group than among those mentioned in the preceding paragraph. Differentiation may, therefore, to some degree prevent the bleeding. One of the common findings in this group is sterility and

there are a few cases of hypomenorrhea. The histologic characteristics are not greatly different from those of the normal early differentiative phase. The number of glands is essentially the same. In this group the glands are more convoluted and the proliferative epithelium changes, under partial corpus luteum effect, to a more columnar differentiative epithelium. The gross thickness of the endometrium is approximately 3 mm. The specimen illustrated (fig. 9) was removed from a patient who complained of sterility and who, following stimulation by corpus luteum, became pregnant. The sterility had existed for five years.

Persistent late differentiative phase. When the degree of ovarian failure is small, that is, when there is nearly a complete luteinizing effect on the endometrium, the histology of the endometrium is also only slightly altered. Differentiation is complete, or nearly so, but clinically there may be some slight abnormality in the menstrual cycle. However, most of the patients menstruate regularly and complain of either sterility or of some symptom suggestive of ovarian failure. Such as endometrium as that under consideration usually will contain six to seven longitudinal glands per low power field. In longitudinal section these glands appear as a sectioned seashell. The glands are lined by columnar differentiated epithelium. Cases of this sort are favorable to treat because only slight stimulation often will correct the dysfunction. Cystic changes often are seen in this type of endometrium and constitute a very definite abnormal finding (fig. 10).

Cystic endometrium. The occurrence of cystic areas in the endometrium is always indictive of failure of corpus luteum and is the first single abnormal feature observed in histologic examination of the endometrium associated with the failing ovary. Previously I have pointed out that these cystic areas are not indicative of hyperplasia but rather are indicative of physiologic senility. Cystic areas are a constant finding in menopausal endometrium and it is known that at menopause the first failure to occur in the ovary is in the corpus luteum. This fact has been definitely established by examination of sections of ovaries of menopausal patients, in which no corpus luteum can be found.

In the young patient, however, the finding of these areas again means exactly the same thing as regards ovarian function and in fact the clinical phenomena are often the same.

Randall and I recently have reviewed a group of cases in which these cystic changes occurred in the endometrium. These results

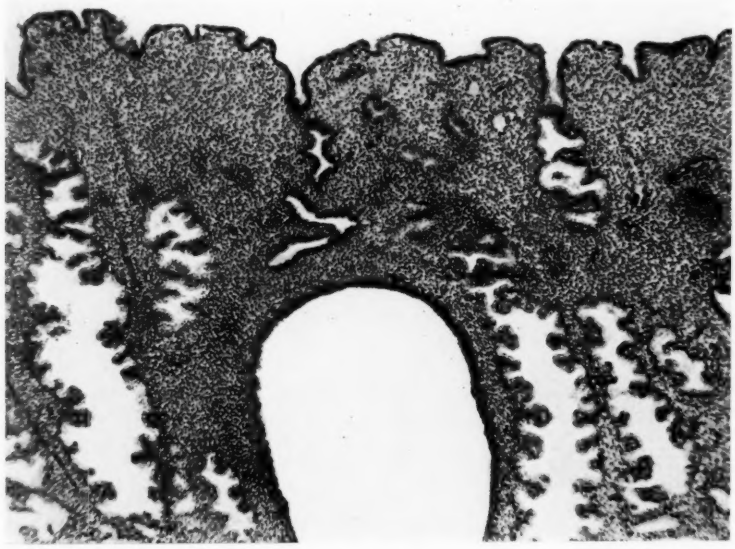

FIG. 10. LATE DIFFERENTIATIVE PHASE OF THE MENSTRUAL CYCLE IN A CYSTIC ENDOMETRIUM

Complete differentiation is evident. There are six to seven longitudinal glands to the low power field but differentiation is complete. The abnormal feature is the presence of cystic areas existing in an endometrium which histologically gives evidence of the nearly complete effect of the hormone of the corpus luteum. Hematoxylin and eosin, ×50.

are shown in table 1. The interesting feature of this problem is in the fact that cystic areas may occur in any phase of the cycle; for example, these areas may be seen in an endometrium that is in the phase of persistent early differentiation and this alone is enough evidence to refute the current belief that the corpus luteum follows an "all or none" law. Since even partial differentiation never occurs in the absence of stimulation by corpus

luteum and since the cystic areas do indicate partial failure of corpus luteum, the occurrence of both partial differentiation and cystic areas together obviously means that a partial corpus luteum effect has been obtained and that various degrees of failure of corpus luteum may exist. It would be highly improbable that the "all or none" principle could be established for this gland of internal secretion when, for example, no other gland is known to obey such a law; for example, any degree of failure may exist in the presence of pituitary, thyroid, adrenal or gonadotropic dysfunction.

TABLE 1

CYSTIC ENDOMETRIUM

CYSTIC ENDOMETRIUM*	CASES	STERILITY		BLEEDING DYSFUNCTION	
		Number	Per cent	Number	Per cent
Early proliferative phase	5	2	40	3	60
Late proliferative phase	8	5	62	4	50
Early differentiative phase	9	7	77	3	33
Late differentiative phase	6	6	100	0	00
Total	28				

* 278 biopsies; 28 cystic.

SUMMARY

The activity of the ovary is reflected in the activity of the endometrium. The normal endometrial cycle can be divided into the menstruating phase, a phase of early proliferation, a phase of late proliferation, a phase of early differentiation, and a phase of late differentiation. The four last named phases correspond roughly to the four weeks of the normal menstrual cycle in the order named. The abnormal endometrial cycles which are owing to abnormal ovarian activity reflect themselves in an arrestment of the cycle in any of the phases named above. The phase of arrestment is called "the peristent phase," and the stage of arrestment depends on the degree and kind of ovarian dysfunction.

The clinical classification of ovarian dysfunction can be divided

into a primary and a secondary group. The primary ovarian dysfunctions are owing to failure in the ovary itself. The secondary dysfunctions are owing to changes in the ovary which accompany or follow failure of the thyroid or pituitary functions. In both groups the histologic manifestations are the same because, as stated, the endometrium reflects only the activity of the ovary.

The study of cystic changes in the endometrium associated with different phases of the cycle indicates that one may separate to some degree the cases of sterility from those of bleeding dysfunction. When cystic changes are present in the proliferative phase of the cycle the tendency is greatest toward a bleeding dysfunction and to a lesser degree toward sterility. On the other hand, when cystic changes are associated with the differentiative phases, the tendency is greatest toward sterility, while the tendency toward bleeding dysfunction is almost entirely absent.

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RAT-BITE FEVER (SODOKU)*

Report of Five Cases

R. S. LEADINGHAM

Atlanta, Georgia

Rat bite fever is a specific infectious disease caused by the Spirillum minus the clinical course of which is characterized by (1) development of a "primary lesion" at the portal of entry, (2) sudden febrile onset and recurring paroxysms of chills and fever, (3) muscular aches and pains, (4) neutrophilic leucocytosis, (5) cutaneous eruption, (6) lymphadenitis and lymphangitis, and (7) varying degrees of prostration.

As its name implies, the illness usually follows the bite of an infected rat. Dogs, cats, and other animals have been known to transmit the infection but the organism appears to be most commonly parasitic in wild rats and mice.

Rodent infection was first discovered in 1887 by Carter[3] who found a spiral organism in the blood of a wild rat and named it Spirillum minor. In 1906 Wenyon,[13] at the Pasteur Institute, and Breinl and Kinghorn[14] described similar organisms which they named respectively Spirochaeta muris and Spirochaeta laverani. In 1916, Futaki[5] and his associates announced their isolation of the Spirochaeta morsus muris from the skin, lymph glands, and blood, in cases of rat bite fever. While there has been some controversy concerning the classification of the organisms described by these and other observers, Robertson,[9] in 1924, suggested the designation Spirillum minus which is generally accepted at the present time.

McDermott[7] notes, as a matter of historical interest, that the

* From the Department of Medicine, Emory University, School of Medicine. Read at the Sixteenth Annual Meeting of the American Society of Clinical Pathologists, Philadelphia, Pennsylvania, June 5, 1937. Received for publication August 6th, 1937.

Spirochaeta laverani was used by Ehrlich in his classical experiments in the chemotherapy of spirochetal infections leading to the discovery of salvarsan.

In pathogenicity the Spirillum minus bears a resemblance to spirochetes and, like them, is destroyed by salvarsan and similar arsenical compounds. While it has been found in secretions from initial lesions and cutaneous eruptions by dark field examination, and in tissues stained by Levaditi's method, intraperitoneal or subcutaneous inoculations of laboratory animals is necessary for its isolation from the patient's blood stream. White mice or rats are most suitable for this purpose. Five-tenths to 1 cc. of whole blood may be used. The organisms may be destroyed by sodium citrate if used as an anticoagulant. Physiological saline is a satisfactory vehicle for transfer inoculations with small amounts of animal's blood.

The Spirillum minus is a broad spiral organism measuring two to five microns in length. It has regular spirals and about one spiral per micron. Some have tapering, and others blunt ends provided with one or more flagella. It may escape the notice of an inexperienced observer in dark field preparations. Once seen, however, identification should not be difficult as I know of no similar organism with which it might be confused. Its movements are very rapid and vigorous, and it darts back and forth across the field pushing the blood cells about so that its presence may be suspected before it is seen. At times it seems to have a spinning motion, especially when it strikes some obstacle in its path. Motility may be retarded by cooling the slide with ice and movements may cease after long exposure to a dark field lamp. During rapid movements, it's body appears to be rigid but, as they become slower it bends and straightens, showing a considerable degree of flexibility. Although the flagella are readily demonstrated in dark field preparations, they are difficult to stain. Adachi,[1] in an interesting study, illustrated with photomicrographs and free hand drawings, described numerous variations in their location, length, type, and number. He noted no relation between the length of the organism, or the number of its windings, and the number of flagella which were present,

singly or in tufts, at one or both ends. Some appeared branched and others united to form a large one. The largest number he counted was seven.

The organisms may be stained with anilin dyes in blood smears fixed with methyl alcohol. A satisfactory method is as follows:

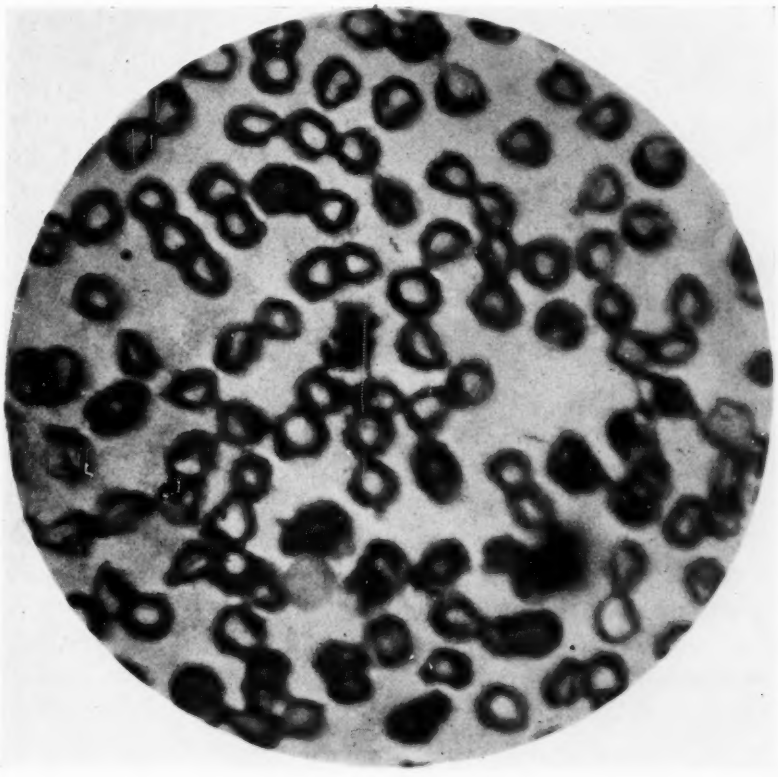

FIG. 1

Smear the blood thinly over the slide and dry it in the air; fix in absolute alcohol for twenty minutes; stain with Giemsa's stain, using 10 cc. of stain in 100 cc. of distilled water to which is added twenty drops of one per cent potassium carbonate for each 10 cc. of solution, for from fifteen to sixteen hours; rinse in distilled water and dry; pass quickly through pure xylol and mount in balsam. They also may be stained, though less satisfactorily, by Fontana's method.

The spirillum minus was isolated by mouse inoculation from three of this series of five cases. In one instance, the organisms appeared in the animal's peripheral blood stream in fifteen days, and, in two cases, they were not found until twenty days after inoculation. Transfer inoculations were followed by the appearance of the organisms as early as the fifth day. At first, only an occasional organism was found by dark field examination, but within two weeks their numbers increased until usually one to four were present in a single field. Thereafter, their numbers gradually decreased until, in two or three months, they were hard to find. I have, however, known an animal to be infective, as long as twelve months.

Most of the animals used have been discarded within six months after transfers, during which time they have shown no ill effects from the infection. One animal, however, died nine months after inoculation and following three weeks of indisposition, loss of weight, alopecia, and severe conjunctivitis. No organisms were found in the blood stream by dark field examination at this time. At autopsy, the lungs contained a large number of circumscribed granulomas and diffuse areas of interstitial infiltration, especially about the larger blood vessels, and the bronchi were filled with a thin exudate containing few round cells. Intra-abdominal lymph nodes were enlarged and contained lesions similar to the granulomas in the lungs. They were composed principally of round cells and large monocytes surrounded by thin margins of connective tissue proliferation. Many showed central areas of necrosis. No giant cells were found. Tissue stained by Giemsa's and Levaditi's methods revealed spiral organisms within the granulomas which, in most instances, were larger than the Spirillum minus found in the blood stream, though in many respects they were strikingly similar. McDermott described in rats carrying the Spirillum minus similar lesions which he thought were "tertiary" or "gummatoid" manifestations of the disease because material from them transmitted the infection to other animals.

Two animals inoculated at the same time as the rat that died were sacrified and showed only moderate infiltration about the larger blood vessels in the lungs.

Tunnicliff,[12] in 1916, however, called attention to the close resemblance, in tissue stains, of the Spirochaeta morsus muris described by Futaki to the streptothrix she isolated from rats suffering from bronchopneumonia, which was similar to the organism Schottmuller, Blake, Tileston, and others found in cases of rat-bite fever and named streptothrix muris ratti. The organism, like the Spirillum minus, appears to be a normal parasite for rats and is closely related to the actinomyces group. Topley[11] and Wilson, commenting on these observations, note the similarity of the Streptothrix muris ratti and the Streptobacillus moniliformis described by Strangeways and Levaditi, Nicolau, and Poincloux, and conclude that these are identical organisms. They suggest for them the name Actinomyces muris.

The Actinomyces muris may cause an acute septicemia or a chronic illness in mice characterized by edematous swelling of the feet and legs, enlargement of the lymph glands, and keratitis. Infection probably occurs as the result of bites, or through contamination with urine, which may contain the organisms. They may be cultivated in serum broth and on Loeffler's blood serum.

It has been assumed that human infection with the Spirillum minus occurs when there are abrasions of the animals' gums which allow their blood to infect the wounds made by their teeth. No organisms have been found in their saliva, but some investigators have found them in the lachrymal fluids of animals suffering from conjunctivitis.

Periodic examinations throughout the lives of animals harboring the organisms, and examinations of the tissues of others sacrificed at stated intervals, might reveal avenues of infection from the gastrointestinal and respiratory tracts and indicate to what extent intercurrent illnesses may contribute to the transmission of the disease.

Guinea pigs inoculated with organisms isolated from each of the three cases in this series developed a febrile illness with alopecia, loss of weight, and death in from four to eight weeks.

Rabbits and monkeys are susceptible to the infection.

The organisms cannot be cultivated in routine culture media.

It is stated that cultures have been obtained in Shimamine's medium, but successive transfers have not been successful.

Although other infections may be transmitted to man by a rat's bite, the Spirillum minus has been identified with a clinical syndrome sufficiently characteristic to permit restriction in the use of the desigation "rat-bite fever" or "sodoku" to the disease caused by this organism.

There are many species of rats to be found in every locality, but among them the Rattus norvegicus[10] is the most vicious and aggressive. This animal is commonly known as the "Norway rat" or "sewer rat" and is the largest of the species. While it seems to prefer underground nesting places, it may be found inside of buildings, even in the upper parts of them, if they are not properly rat-proofed. It frequently invades the sleeping quarters of poorly constructed dwellings. In two instances, here recorded, the patients were bitten while asleep in bed and the other occupants of the room saw the animals jump from the beds and trot across the floor. They are not as agile as other species of rats but are more aggressive and fearless. They fight not only among themselves but will frequently turn upon a cat or dog and, when attacked by man, will often stand and fight until killed.

From 1 to 25 per cent of wild rats examined by various investigators have been found to harbor the Spirillum minus, and Francis[4] found the organisms in sixty-five of a lot of one hundred and fifty white mice delivered to him for laboratory purposes.

Sodoku was recognized in Japan a long while before the discovery of the Spirochaeta morsus muris and Miyake[8] described the illness in 1901.

The disease is now known to be world-wide and there is little doubt that the number of cases reported does not indicate the frequency of infection. Bayne-Jones,[2] in 1931, reviewed eighty-one cases in the United States in five of which the Spirillum minus was isolated. Three of this series were mentioned in a previous article[6] and are included in his review. They are reported here in more detail with two more recent cases.

Case 1. W. M. H. No. 25264. University student, white male, age 20. The patient was bitten on his wrist by a large gray rat which he had cornered

in the biological laboratory and was trying to kill. The wound was superficial and bled very little. It was painted with iodine and healed in four days. Thirty-six days later, he was taken ill with fever (102°F.), chilly sensation, and aching pains in his left shoulder. He called a doctor who made a diagnosis of malaria and prescribed quinine, 15–20 grains daily for three days, and then 5 grains daily for one week. His fever was continuous for 3 days after onset and on the 4th day there was a circumscribed area of inflammation resembling a small boil on his wrist where the rat had bitten him. It later turned blue and persisted for seven days. The left axillary lymph nodes became swollen and he had aching pains in his left arm and shoulder. His doctor then gave him an intravenous injection of quinine.

On the 8th and 9th days of his illness he had afternoon fever of 103°F. and 104°F. He had no fever on the 10th day. On the 11th, 12th, 13th, 14th, 15th, and 16th days there was again fever of 103°–104°F. There was none on the 17th day. On the 18th day he had a temperature of 104°F. and red streaks extended up his arm. On the 19th day his temperature was 105°F. and pink erythematous spots appeared over both arms, abdomen and chest, and on his right temple which were described as about 1 cm. in diameter. None were present the next day, the 20th day of illness, 56 days after the bite, when he was admitted to the hospital.

Examination of his blood showed no malarial parasites and a leucocytosis of 18,200 with 80 per cent neutrophiles. Blood Wassermann was not made.

A diagnosis of rat-bite fever was made from the history of his illness. White mice were inoculated with his blood and 0.3 gram salvarsan administered intravenously. Complete recovery followed.

Spirillum minus appeared in the animal's blood twenty days after inoculation.

Case 2. W. M. H. No. 27224-27642. White school boy, age 11.

The patient was carrying a dinner pail to his brother in a field, near a barn, when five large rats, crossing the road along which he was walking, attacked him. One got a deep hold on his forefinger which could not be released until it was killed.

Eleven days later, a physician began antirabic treatment. After the fifth dose, 16 days after the bite, the child went to bed with a temperature of 103.5°F. His right axillary lymph nodes were enlarged. Ttreatment was omitted the next day and his temperature was normal. Five more daily doses of anti-rabic vaccine were given when he again had a temperature of 104°F. The doctor skipped two days and then gave five more injections when the patient became ill again with a fever lasting three days. Both right and left axillary lymph nodes were enlarged and tender.

Thinking that these febrile attacks were due to the anti-rabic vaccine, the physician discontinued treatment. Twenty-seven days after the boy was bitten, the doctor wrote that the right axillary and cervical lymph nodes were

enlarged and tender. Areas about these nodes also appeared inflamed and the boy's temperature was 101°F.

The patient was admitted to the hospital five days later, the 32nd day of his illness and 48 days after the bite, with a temperature of 99.2°F., pulse 124, respiration 24. His cheeks were flushed and right axillary and cervical lymph nodes were enlarged and tender. There was a large bluish red erythematous area in the right axilla. The wound on his finger was not inflamed.

His blood count showed 90 per cent hemoglobin (Dare); 4,580,000 red blood cells; 8,400 leucocytes with 79 per cent neutrophiles. Blood Wassermann was positive. White mice were inoculated.

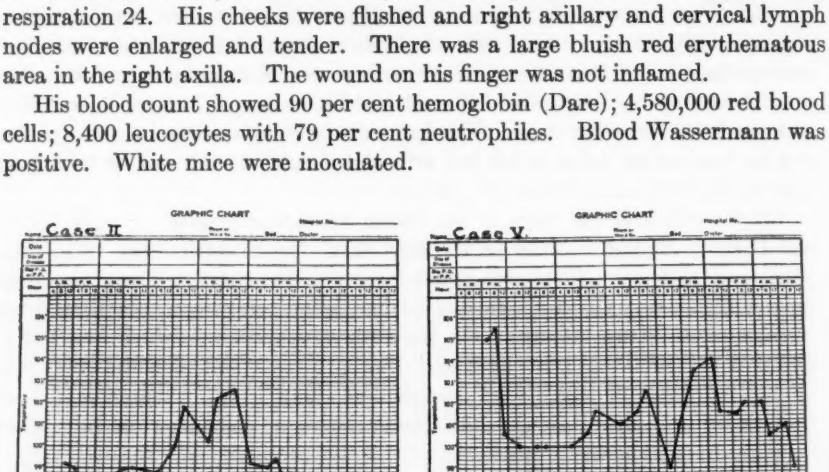

CHART 1. CHARACTERISTIC HYPERTHERMIC EPISODE IN RAT-BITE FEVER

On the 3rd hospital day, his right arm was covered with a purple erythematous rash and was very painful. His temperature was 101°F., pulse 128. On the 4th hospital day, his fever reached 102°F., pulse 130. He was given salvarsan 0.1 gram intravenously, and six hours later was nauseated and vomited. The next day he felt better, and the day following his temperature was normal and his pulse varied from 80 to 104. On the 9th hospital day, he was again given salvarsan 0.1 gram, and this dose was repeated six days later. He was dismissed after sixteen days, during which time he had gained three and one-half pounds in weight.

Rat-bite fever organisms appeared in the blood of all inoculated animals fifteen days after inoculation.

Thirty days after dismissal, he was readmitted with his right axillary and cervical lymph nodes enlarged and painful. He had had some elevation of temperature at irregular intervals since leaving the hospital. His temperature on admission was 99°F., pulse 94. His weight, sixty pounds, was the same as when he was dismissed. He was given 0.2 gram salvarsan, which was followed in four hours by nausea and vomiting. His temperature rose on the 2nd and 3rd day to 99.8°F., and on the 4th day, it reached 101.6°F.; pulse 130. Salvarsan 0.2 gram was repeated. On the 10th hospital day, his blood count showed 90 per cent hemoglobin (Dare); 4,140,000 red blood cells; 10,500 leucocytes, with 52 per cent neutrophiles. Blood Wassermann was positive. He was given then another injection of 0.2 gram salvarsan, and again on the 13th hospital day. Lymph nodes were no longer swollen or painful. Up to this time, his temperature varied from 98 to 99.2°F., except for the acute rise soon after admission.

On the 16th day, he was given another injection of 0.2 gram salvarsan, and thereafter there was little variation of pulse or temperature until the 28th day, when he was dismissed.

Father's and mother's blood Wassermann were negative and the child had no evidence of syphilis.

Case 3. G. H. No. 20074. Negro male child, age 10.

Bitten on the nose by a rat while asleep in bed. The wound healed promptly. Two weeks later his nose began to swell, and there developed a painless swelling of the left side of his face extending from the mid-line of his chin beyond the angle of his jaw. His left eyelid became swollen and the left infraorbital lymph node enlarged to the size of a hickory nut.

On admission to the hospital, his temperature was 103.4°F.; pulse 110. For two weeks he had an irregular fever, at times reaching 104°F., with three short remissions. His urine contained albumen without red blood cells or casts. His blood count showed 5,000,000 red blood cells with 85 per cent hemoglobin (Tallquist), and 10,400 leucocytes with 70 per cent neutrophiles.

White mice were inoculated and 0.3 gram neoarsphenamine was given intravenously.

His temperature subsided promptly and he was dismissed two days later.

The animals died ten days after inoculation without any organisms being found in blood or peritoneal fluid.

Case 4. J. R. W. Zebulon, Ga. White farmer, age 79.

The patient had been in poor health for some time, suffering from arteriosclerotic heart disease.

One morning, as he opened the door of a crib back of his house, a large rat jumped upon him and ran up his coat. As he grasped the animal, it caught his left forefinger with its teeth and had to be killed to loosen its hold. He applied iodine to the wound, and, on the 4th day, it was healed. That afternoon,

he had a hard chill followed by fever of 103°F. His temperature was normal next morning, but, in the afternoon, he had another chill and his temperature reached 102.5°F. The next afternoon, he had a lighter chill followed by temperature of 102°F. He became very weak and complained of aching in his right shoulder, and three days later, he could not use his right arm because of the severity of the pain. He felt somewhat better the next two or three days and then had severe aching in both legs. He grew steadily weaker and had another chill followed by fever.

When seen, ten days after the rat bite, he was moaning with discomfort whenever moved. His temperature was normal, pulse rapid, and moist râles were present over the bases of both lungs. There was an area of bluish discoloration on his left forefinger where he had been bitten. Lymph nodes were not enlarged. There was no evident lymphangitis and no skin eruption.

Blood count showed 16,500 leucocytes with 80 per cent neutrophiles. Blood cultures were negative. Blood Kahn was negative. Mice were inoculated and died a week afterwards without showing organisms in their blood streams or peritoneal fluid. The cause of their death was not determined.

Intravenous injection of 0.45 gram neoarsphenamine was followed by clinical improvement, and he had no more chills or fever.

His physician reported that death occurred about three weeks later and was probably due to a cerebral accident.

Case 5. G. H. No. 112051. White male child, age 7.

The patient was bitten on the left ear and neck by a rat while asleep in bed. The wounds were dressed by a physician and promptly healed. Two weeks later, he began to have daily chills and fever. The original wounds were inflamed and surrounded by an area of discoloration which extended to his clavicle. Later, a purple eruption covered his entire body and was present throughout his illness of nine weeks, usually more pronounced when his fever was high. The glands of his neck and groin became enlarged and tender, and he suffered severe aching pains over his entire body. Shortly before admission to the hospital, on the 66th day of illness, and 80 days after the bite, chills and fever occurred at lengthening intervals.

On admission the cervical, axillary, and inguinal lymph nodes were enlarged and tender, and there were pink erythematous patches over his face and chest.

His temperature was 105°F., and, four hours later, was 105.6°F.; pulse, 145. In another four hours, his fever dropped to 100.6°F. and remained at 100°F. for 24 hours. Then there was a gradual rise to 102.6°F. in thirty-six hours, with a fall to 99°F. in twelve hours, that was followed by a sharp rise to 104°F. in twenty hours. It had begun to fall by lysis when white mice were inoculated and 0.15 gm. neoarsphenamine administered intravenously. A blood culture was negative. Wassermann was negative. His blood count showed 19,100 leucocytes with 76 per cent neutrophiles. His temperature was normal two

days later when 0.3 gm. neoarsphenamine was again given. He was dismissed on the 10th hospital day with temperature 98°F. and pulse, 80.

All animals showed the Spirillum minus in their peripheral blood streams twenty days after inoculation.

SUMMARY

The five cases of rat-bite fever in this series followed the bites of rats with the described characteristics of the Rattus norvegious.

Two patients were bitten on their fingers, one on the wrist, one on the nose, and one on the ear and neck. In all cases the wounds healed promptly.

Incubation periods varied from four to thirty-six days. "Primary lesions," induration of original wounds, occurred in three instances with the onset of chills and fever. Lymphadenitis was present in four cases. Cutaneous eruptions occurred in three cases. Leucocytosis varied from 8,400 to 19,100 with neutrophils from 52 per cent to 80 per cent. Blood cultures taken in two cases were negative. Blood Wassermann or Kahn tests were negative in two cases and Wassermann was strongly positive in one. All patients complained of aching pains in muscles and joints.

The clinical course of the illness was characterized, in each instance, by recurring paroxysms of chills and fever at about five days intervals. In two cases, the hyperthermic episodes observed in the hospital showed a septic type of fever lasting forty-eight and ninety-six hours, falling, in one instance, by crisis, and in the other, by lysis.

Three patients were each given one injection of salvarsan or neoarsphenamine, one had two, and one, seven injections.

The characteristic clinical course of an illness following a bite by a wild rat, or other susceptible animal, should warrant the diagnosis of rat-bite fever and the use of arsenicals before organisms are isolated by animal inoculation. Prompt alleviation of symptoms should follow their administration if the illness is caused by the Spirillum minus.

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PATHOLOGY OF THE LUNGS AND OTHER ORGANS IN SILICOSIS*

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The present study was conducted to obtain information on the gross and histological findings in the lungs, extrapulmonary organs and tissues in a group of silicotics coming to autopsy in the hospitals of the Veterans' Administration.

While the pathology of silicosis of the respiratory tract and lymph nodes is well known, little is known of the extrapulmonary changes. It was thought, therefore, that a study of the morbid changes in a group of silicotics might furnish the desired information.

AUTOPSY MATERIAL

The autopsy material which forms the basis of this study consists of twenty-three cases, classified as follows:

	cases
Silicosis	5
Silicosis with infection	3
Silicosis with tuberculosis	8
Silico-tuberculosis	6
Asbestosis	1
Total	23

The patients had been treated in a number of the hospitals of the Veterans' Administration and the postmortem examinations in the majority of instances were performed by full-time pathologists of the Veterans' Administration. Several of the postmortem examinations, including histological studies of the lungs and other organs, were done by Dr. Leroy U. Gardner of Saranac Lake, New York.

* From the Medical and Hospital Service, Veterans' Administration. Received for publication June 16th, 1937.

TABLE 1

CASES OF SILICOSIS WITH PATHOLOGICAL CHANGES IN THE LUNGS AND OTHER ORGANS AS SEEN AT AUTOPSY

CASE	INITIALS	OCCUPATION	LENGTH OF EXPOSURE (YRS.)	DURATION (YRS.)	TYPE OF SILICOSIS	AGE AT DEATH (YRS.)	LUNGS	PLEURA	HEART AND BLOOD VESSELS	KIDNEYS	OTHER ORGANS OF UROGENITAL TRACT	LIVER	SPLEEN	LYMPH NODES	GASTROINTESTINAL TRACT	MISCELLANEOUS
1	W. H.	Iron moulding	40	16	S1	72	Fibro-nodular silicosis, bilateral, especially in upper lobes Metastatic carcinoma, bilateral Chronic passive congestion, bilateral Emphysema	Chronic adhesive pleurisy, bilateral	Cardiac hypertrophy, with marked dilatation Fibrosis of myocardium Arteriosclerosis of aorta Arteriosclerosis of coronary arteries General arteriosclerosis	Retention cysts, bilateral	Carcinoma of the prostate Chronic cystitis Vesical calculus	Chronic passive congestion		Metastatic carcinoma, retroperitoneal lymph nodes Chronic lymphadenitis, tracheobronchial, peritoneal, pancreatic lymph nodes	Gastric ulcer Duodenal ulcer	
2	J. S.	Anthracite coal mining	20	2	S2	40	Fibro-nodular silicosis, bilateral Chronic passive congestion, lower lobe, left lung	Hydrothorax, bilateral Chronic adhesive pleurisy, bilateral	Cardiac hypertrophy Fibrosis of myocardium Infarct of heart	Infarct		Chronic passive congestion	Infarct	Chronic lymphadenitis, tracheobronchial lymph nodes	Chronic cholecystitis	
3	L. J.	Granite cutting	12	8	S2	44	Fibro-nodular silicosis, bilateral Chronic passive congestion	Chronic adhesive pleurisy, bilateral	Arteriosclerosis of aorta	Chronic passive congestion	Hypertrophy of prostate	Primary carcinoma Atrophic cirrhosis	Chronic splenitis	Chronic lymphadenitis, tracheobronchial lymph nodes	Ruptured esophageal varices with hemorrhage	
4	A. D.	Stone cutting	7½	3	S2	37	Fibro-nodular silicosis, bilateral	Hydrothorax, left Chronic adhesive pleurisy, apical, bilateral				Hypertrophic cirrhosis	Chronic splenitis	Chronic lymphadenitis with necrosis and caseation, tracheobronchial lymph nodes		Abdominal ascites
5	J. Z.	Anthracite coal mining	3	3	S3	37	Fibro-nodular silicosis, bilateral Pulmonary edema	Chronic adhesive pleurisy, bilateral	Cardiac hypertrophy Arteriosclerosis of coronary arteries Arteriosclerosis of aorta	Infarct		Chronic hepatitis	Infarct	Chronic lymphadenitis, tracheobronchial lymph nodes		

SILICOSIS

The autopsy material of silicosis consisted of five cases. The patients had been classified as first, second, and third degree silicosis. The gross pathological findings in many instances confirmed by histological studies may be seen by referring to table 1.

The length of exposure of these cases varied from three years to as long as forty years. The occupations varied as will be seen by referring to the table. The duration of the silicotic disease varied from a minimum of two years to a maximum of sixteen years. The ages at death in four instances were between thirty-seven and forty-four years, and in one instance the age at death was seventy-two years.

Lungs. It is of interest to note that in the case of an iron molder with an exposure of forty years and a total of sixteen years' duration of silicosis, the nodulations were smaller than in instances in which there were shorter periods of exposure to siliceous dust and a shorter duration of the disease. In four of the five cases chronic passive congestion or edema of the lungs was found.

Pleurae. In all five cases the pleurae were adherent to the upper lobes of the lungs, especially in the apices.

Emphysema. Compensatory emphysema was present in one case (W. H.), with a history of exposure to silica for forty years, and a history of silicosis of sixteen years' duration. This patient was seventy-two years of age at the time of death.

Cardiovascular diseases. Of the five silicotics, three gave evidence of cardiac hypertrophy. One of the three cases had a coexisting cardiac dilatation; two of the five cases gave evidence of fibrosis of the myocardium; two showed the presence of arteriosclerosis of the coronary arteries; and three gave evidence of arteriosclerosis of the aorta.

Diseases of the liver. All five cases gave evidence of pathological changes of the liver. In two, there was chronic passive congestion; in one there was atrophic cirrhosis and primary carcinoma; one showed the presence of chronic hepatitis; and in one a hypertrophic cirrhosis was noted.

Diseases of the spleen. One of the five cases had a normal spleen; infarcts were found in two cases; and two cases gave evidence of chronic splenitis.

Lymph nodes. In the five cases of silicosis, the tracheobronchial lymph nodes were either enlarged and pigmented, or were found to be smaller than normal. They were dark gray in color and fibrotic.

SILICOSIS WITH INFECTION

In silicosis, respiratory infections are frequently encountered due to a lowered immunity of the patient. While tuberculosis is the most frequent infection, there are other conditions which may complicate silicosis, such as pneumonia, lung abscess, bronchiectasis, influenza, empyema, and conditions due to the

TABLE 2

CASES OF SILICOSIS WITH INFECTION SHOWING PATHOLOGICAL CHANGES IN THE LUNGS AND OTHER ORGANS AS SEEN AT AUTOPSY

CASE	INITIALS	OCCUPATION	LENGTH OF EXPOSURE (YRS.)	DURATION (YRS.)	TYPE OF SILICOSIS	AGE AT DEATH (YRS.)	LUNGS	PLEURA	HEART AND BLOOD VESSELS	KIDNEYS	OTHER ORGANS OF UROGENITAL TRACT	LIVER	SPLEEN	LYMPH NODES	GASTROINTESTINAL TRACT	MISCELLANEOUS
6	P. S.	Employed in brickyard	Unknown	13	S2 and infection	46	Fibro-nodular silicosis, bilateral Pyothorax, left Pneumothorax, spontaneous, left	Chronic adhesive pleurisy, bilateral Emphyema, left	Cardiac dilatation	Chronic passive congestion		Chronic passive congestion	Chronic splenitis Chronic passive congestion			
7	W. J. S.	Lead and silver mining	8	1	S3 and infection	55	Fibro-nodular silicosis, bilateral	Chronic adhesive pleurisy, bilateral	Cardiac hypertrophy with dilatation Chronic adhesive pericarditis	Chronic nephritis		Chronic hepatitis			Chronic adhesive peritonitis	
8	F. C.	Rock drilling	Unknown	Unknown	Conglomerate silicosis and infection	41	Fibro-nodular silicosis, bilateral Bronchopneumonia, bilateral Bronchiectasis, bilateral	Chronic adhesive pleurisy, bilateral	Cardiac dilatation, right Pericarditis with effusion							

fuso-spirochetal organisms. The aerobic organisms, which may be the cause of the complicating infections of silicosis, are the staphylococcus, streptococcus, Friedlander bacillus, colon bacillus, etc.

Proske and Sayers[1] state that the mode of infection in the lungs may be by aspiration, through the lymphatics, the blood stream, as the result of an embolus, or by introduction directly into the chest by a stab or gunshot wound.

These observers describe the following mechanism of infection, which may complicate silicosis: The large quantity of dust inhaled is contaminated with infectious material from the teeth, gums, or other sources. The finer dust particles, which are not eliminated by the respiratory mechanism, lodge in the alveoli, where they cause a catarrhal inflammation and hence establish favorable conditions for the growth of the organisms which they carry. The monocytes engulf some of the dust particles and bacteria and carry them to the lymph nodes where the heavily laden monocytes disintegrate and release the organisms which cause the complicating infection.

In table 2 are listed three cases of silicosis with infection, in which are shown the pathological changes in the lungs as well as in other organs. In case six there is evidence of coexisting pyothorax and empyema. In case seven, chronic adhesive pericarditis complicates the silicotic disease. In case eight, bronchopneumonia, bronchiectasis, and pericarditis with effusion are the complicating infections.

These cases gave evidence of cardiac involvement; two showed the presence of cardiac dilatation, and the third showed the presence of cardiac hypertrophy and dilatation.

One of the patients, forty-six years of age at the time of death, showed the presence of chronic passive congestion of the kidneys, liver, and spleen.

The second patient, fifty-five years of age at the time of death, showed the presence of cardiac hypertrophy and dilatation, also adhesive pericarditis, chronic nephritis, chronic hepatitis, and chronic adhesive peritonitis.

The third case, a rock driller, forty-one years of age at the time of death, showed evidence of confluent nodulations of the lungs, bronchiectasis, bronchopneumonia, and in addition dilatation of the right heart.

COMMENT

The findings in these three cases indicate the types of infection which may complicate silicosis and portray the coexisting pathological changes in the lungs, as well as in the extrapulmonary tissues, which may result from silicosis with infection. The findings indicate that cardiac hypertrophy or dilatation, or a combination of these two abnormalities of the heart, are frequent complications of silicosis and play an important part in the death of patients with silicotic disease.

SILICOSIS AND TUBERCULOSIS

The question of the causal relationship of silicosis to tuberculosis has received careful study by laboratory workers as well as clinicians, with the result that the consensus is that there is a relationship between the two diseases.

Gardner[2] conducted a series of experiments on guinea pigs, infecting them first with tubercle bacilli and then exposing certain of the animals to quartz, silica, and carborundum dust until the animals died. In the case of the animals inoculated with tubercle bacilli only, the lesions did not spread, but healed, and eventually all evidence of tuberculosis completely disappeared. Where dusting was resorted to subsequent to the healing of the tuberculous foci, it was shown that silica was the most potent, carborundum next, and granite dust the least potent factor in reactivating the healed tuberculous foci.

In the undusted, control animals the tubercles completely disappeared after two or three years. Where the animals were exposed to prolonged inhalation of silica dust, reactivation of the primary tubercles began after two or three months.

Microscopically, it was found that the primary tubercles, containing considerable amounts of dust, continued to spread. The mechanism responsible for the spread of the tuberculous infection is not understood. Whether the alkaline body fluids are able to dissolve the silica and this, in turn, acting directly on the existing tubercle bacilli causes them to multiply, or whether the silica or other dusts first react upon the tissue cells and thereby cause an alteration of the metabolism with the formation of substances favorable to the growth of the tubercle bacilli, is not definitely known.

Experimentally, in silicotic animals, subsequent infection with attenuated tubercle bacilli tends to produce a rapid form of the disease, terminating fatally within three or four months. The non-silicotic controls, on the other hand, practically never die from tuberculosis.

In the test tube, silica, when added to artificial media, definitely accelerates the initial lag before tubercle bacilli begin to

grow. This can be explained by its buffering effect, although other factors may be involved, according to Gardner.[3]

In the body of an animal infected in one groin with silica and in the other with aluminum oxide or some other dust and then inoculated intravenously with tubercle bacilli, subsequent examination shows a great many more microorganisms in the local silicotic lesions than in those produced by the aluminum oxide. Kettle believes that the specific necrosis produced by the silica offers a particularly favorable environment for the growth of tubercle bacilli.

In the human being, tuberculosis and silicosis may coexist as more or less separate entities. The tuberculous disease may complicate any of the stages of the silicotic condition. There is another type of the combined disease in which the lung changes are so extensive that it is impossible to determine a separate background for either condition (silico-tuberculosis).

Gardner[4] is of the opinion that certain changes in the body are probably more important than alterations of the bacilli to account for the predisposition of silicotics to tuberculosis. He considers this problem under three headings:

(1) Lymphatics obstruction preventing elimination of the tubercle bacilli from the lungs.

(2) Alteration of the immune state.

(3) Silicotic necrosis as a favorable medium for the growth of the tubercle bacilli.

Gardner holds that tuberculosis may exist in latent form before exposure to silica occurs, and that such latent tuberculous foci may be reactivated and may give rise to progressive tuberculosis under the influence of inhaled silica. It is Gardner's opinion that much of the chronic upper lobe tuberculosis found in silicotics originates in this manner.

When apical tuberculosis is reactivated it causes a chronic type of silico-tuberculosis which slowly progresses downward. Cavity formation may not occur for many years. Intrabronchial spread to remote portions of the lung does not seem to be the common sequel that it is as a complication of non-silicotic tuberculosis.

Tubercle bacilli are difficult to detect even with guinea pig inoculation, until the disease has progressed for many years.

Mid, or lower lung, tuberculosis is more common in silicotics than in normal persons. Such localization occurs in persons who acquire their adult exogenous tuberculous infection after entering the silica industry, for in them the usual evidence of an old apical scar is lacking. Such disease is often chronic in its course, extending locally through the air spaces without much generalized dissemination to other parts of the lung. Ultimately it may break down, form localized cavitations, and spread.

Another type of tuberculosis, which may be seen in silicotics, is an acute form of the disease either miliary or bronchopneumonic in character. In the usual slowly progressive type of silicosis with advanced nodulation, tuberculous infection of the lungs may spread rapidly.

Gardner is of the opinion that the silicotic is more susceptible to tuberculosis than is the normal person, and that the degree of susceptibility increases with the amount of silicotic disease present. Tuberculosis behaves atypically in the presence of silicosis; it is usually chronic in its course, often without the ordinary symptoms of intoxication, and may be carried for years without serious impairment of working capacity. The sputum may be scanty or absent and the detection of bacilli may not be possible until very late in the disease. Tuberculosis in the silicotic does not necessarily progress without interruption to a fatal outcome; under proper treatment, arrest is possible and many of the lesions seem to retrogress when they are followed in serial x-ray films.

AUTOPSY MATERIAL OF SILICOSIS WITH COEXISTING TUBERCULOSIS

A study of the autopsy material of eight cases of silicosis with coexisting pulmonary tuberculosis showed that the age at the time of death of the patients varied from forty-one years to seventy years. Five of the patients were in the fifth decade, two in the sixth decade, and one in the eighth decade of life at the time of death.

The length of exposure varied from four years to twenty-one years. The duration of the combined silicotic and tuberculous disease ranged from one year to eight years.

Tuberculosis coexisted with first degree silicosis in one case; with second degree silicosis in four cases; and with third degree silicosis in three cases.

The tuberculous disease was found in all lobes in three instances and in five instances the lobes affected were as follows: In one case the right upper and middle lobes; in one case the right upper and middle lobes, as well as the left upper lobe; in one case the right and left upper lobes; in one case the right and left lower lobes; and in one case the right and left upper and left lower lobes were affected.

The following were the principal associated diseases noted in the eight cases:

Pleurae. Seven of the eight cases gave evidence of chronic adhesive pleurisy; empyema was present in one; bilateral hydrothorax was noted in one; and one showed a unilateral pneumothorax.

Heart and blood vessels. Four of the eight cases gave evidence of cardiac dilatation; in two the dilatation was in combination with cardiac hypertrophy. In one case cardiac hypertrophy was present alone. Arteriosclerosis of the aorta was noted in five instances; pericarditis in three; and in one case fibrosis of the myocardium was noted.

Kidneys. Acute and subacute nephritis were present in one case each. Chronic passive congestion was noted in one instance. Healed tuberculosis of the kidneys was present in one case.

Liver. Chronic passive congestion of the liver was noted in three instances. Healed tuberculosis was present in one instance.

Lymph nodes. In four instances the tracheobronchial lymph nodes were involved; in two of these cases caseation was present.

COMMENT

A study of the postmortem findings of the eight cases of silicosis with coexisting pulmonary tuberculosis disclosed the fact that the latter infection may complicate silicosis in any stage of the silicotic disease. It may attack the lower lobes as well as the upper lobes. The particular lobes affected depend upon whether the tuberculous disease is a reactivated old focus or whether the source of the infection is exogenous. The upper lobe disease is usually the result of a reactivation of an old tuberculous focus; lower or middle lobe disease is usually exogenous in origin.

The extent of the tuberculous involvement as well as the toxicity depend upon the dosage of the bacillary infection and the immunological response of the patient. According to Cummins[5] the extent of the infection is the result of the accumulation of inhaled tubercle bacilli in the lungs due to blocked lymphatic

TABLE 3

CASES OF SILICOSIS WITH TUBERCULOSIS SHOWING PATHOLOGICAL CHANGES IN THE LUNGS AND OTHER ORGANS AS SEEN AT AUTOPSY

CASE	INITIALS	OCCUPATION	LENGTH OF EXPOSURE (YRS.)	DURATION (YRS.)	TYPE OF SILICOSIS	AGE AT DEATH (YRS.)	LUNGS	PLEURA	HEART AND BLOOD VESSELS	KIDNEYS	OTHER ORGANS OF URO-GENITAL TRACT	LIVER	LYMPH NODES	SPLEEN	GASTROINTESTINAL TRACT	MISCELLANEOUS
9	J. T. C.	Granite cutting	12	3	S1 and tuberculosis	70	Silicosis bilateral Chronic pulmonary tuberculosis with cavitation, right and left upper and left lower lobes Bronchopneumonia, bilateral Bronchiectasis, bilateral Emphysema, right lung	Emphyema, left	Arteriosclerosis of the aorta	Subacute nephritis		Chronic passive congestion				
10	I. H. T.	Pottery work	9	1	S2 and tuberculosis	43	Fibro-nodular conglomerate silicosis, bilateral, with caseation Chronic pulmonary tuberculosis with cavitation, bilateral, all lobes Tuberculous bronchopneumonia	Chronic adhesive pleurisy, bilateral	Cardiac dilatation, right heart				Chronic lymphadenitis, with caseation, tracheobronchial lymph nodes			
11	W. B. M.	Coal mining	Unknown	1	S2 and tuberculosis	44	Fibro-nodular silicosis, bilateral Chronic pulmonary tuberculosis, right upper and middle lobes Atelectasis	Chronic adhesive pleurisy Hydrothorax, bilateral	Cardiac dilatation Acute fibrinous pericarditis			Chronic passive congestion		Chronic passive congestion		Abdominal ascites
12	E. E.	Pottery work	21	8	S2 and tuber-	45	Fibro-nodular silicosis, bilateral	Chronic adhesive pleurisy, bilat-	Chronic adhesive pericarditis	Acute nephri-	Hyper-trophy		Chronic lympha-			

							Atelectasis									
12	E. E.	Pottery work	21	8	S2 and tuberculosis	45	Fibro-nodular silicosis, bilateral Chronic pulmonary tuberculosis with cavitation, right and left upper lobes and right middle lobe	Chronic adhesive pleurisy, bilateral Pneumothorax, right	Chronic adhesive pericarditis Arteriosclerosis of aorta	Acute nephritis	Hypertrophy of prostate		Chronic lymphadenitis, tracheobronchial lymph nodes			
13	S. J. H.	Quartz quarrying	15	2	S2 and tuberculosis	41	Fibro-nodular silicosis, bilateral Chronic pulmonary tuberculosis with cavitation, bilateral, all lobes Chronic passive congestion, lower lobe, left lung	Chronic adhesive pleurisy, bilateral	Arteriosclerosis of ascending aorta					Chronic passive congestion		
14	F. R.	Sand blasting	4	1	S3 and tuberculosis	47	Fibro-nodular silicosis, bilateral Chronic pulmonary tuberculosis with cavitation, right and left lower lobes Emphysema, bilateral Chronic passive congestion	Chronic adhesive pleurisy, bilateral	Cardiac hypertrophy with dilatation Fibrosis of myocardium	Hypernephroma, right kidney Chronic passive congestion Healed tuberculosis		Chronic passive congestion Healed tuberculosis	Chronic lymphadenitis, tracheobronchial lymph nodes	Chronic passive congestion Tuberculosis of spleen		
15	W. G. H.	Quartz quarrying	18	6	S3 and tuberculosis	55	Fibro-nodular silicosis, bilateral Chronic pulmonary tuberculosis, right and left upper lobes, with cavitation, left upper lobe Emphysema, right and left lower lobes	Chronic adhesive pleurisy, bilateral	Cardiac hypertrophy Sub-acute fibrinous pericarditis Arteriosclerosis of aorta				Chronic lymphadenitis, with caseation, tracheobronchial lymph nodes		Ulcer of cecum and stricture of descending colon	Tuberculous meningitis

TABLE 3—*Concluded*

CASE	INITIALS	OCCUPATION	LENGTH OF EXPOSURE (YRS.)	DURATION (YRS.)	TYPE OF SILICOSIS	AGE AT DEATH (YRS.)	LUNGS	PLEURA	HEART AND BLOOD VESSELS	KIDNEYS	OTHER ORGANS OF UROGENITAL TRACT	LIVER	LYMPH NODES	SPLEEN	GASTROINTESTINAL TRACT	MISCELLANEOUS
16	G. C.	Metal mining	21	2	S3 and tuberculosis	55	Fibro-nodular silicosis, bilateral Chronic pulmonary tuberculosis, bilateral, all lobes Chronic passive congestion, right and left lower lobes	Chronic adhesive pleurisy, bilateral	Cardiac hypertrophy with dilatation Arteriosclerosis of aorta							

drainage caused by extensive silicotic fibrosis in the tracheobronchial lymph nodes as well as in the lung tissue.

The degree of the immunological response of the host is the same as is found in simple tuberculosis. Whether or not there is a chemical action of silica upon the pulmonary or lymphatic tissue or upon the tubercle bacilli is not altogether clear,—both such effects have been seen in laboratory animals as well as in cultural experiments.

The principal coexisting diseases found at autopsy were cardiac dilatation alone or combined with cardiac hypertrophy. Accompanying the heart disease was chronic passive congestion in a number of organs. Arteriosclerosis of the aorta was another finding.

SILICO-TUBERCULOSIS

As previously stated, silico-tuberculosis is the classification used for cases which give evidence of both silicosis and tuberculosis and in which the two conditions are so extensive and intimately associated as to make it impossible to determine a separate background for each condition.

Six cases were classified as having silico-tuberculosis. Three of the veterans had been engaged in metal mining; one was an anthracite coal miner; one was a stone cutter; and one had been engaged in granite cutting.

The data showing the length of exposure, the duration of the silico-tuberculosis, also the age at death, were not characteristic.

A study of the sites of involvement did not disclose any characteristic or pathognomonic findings, with the exception that as a general rule there was greater involvement of the lungs in cases of silico-tuberculosis than in those classified as silicosis with tuberculosis.

Pleurae. All six cases gave evidence of bilateral pleural adhesions. In one case there was evidence of bilateral hydrothorax in addition to the adhesive pleurisy.

Cardiovascular system. Four of the six cases gave evidence of cardiac hypertrophy and dilatation. Two gave evidence of cardiac dilatation without any sign of hypertrophy. One case gave evidence of fibrosis of the myocardium; and one showed the presence of chronic myocarditis. One showed the presence of arteriosclerosis of the aorta. One case gave evidence of fibrinous pericarditis. In reviewing the above data one is impressed with the frequency of cardiac hypertrophy and dilatation.

Further study of the data in table 4 showed the presence of chronic passive congestion of various organs,—this was no doubt the result of cardiac decompensation.

TABLE 4

CASES OF SILICO-TUBERCULOSIS SHOWING PATHOLOGICAL CHANGES IN THE LUNGS AND OTHER ORGANS AS SEEN AT AUTOPSY

CASE	INITIALS	OCCUPATION	LENGTH OF EXPOSURE (YEARS)	DURATION (YEARS)	TYPE OF SILICOSIS	AGE AT DEATH (YEARS)	LUNGS	PLEURA	HEART AND BLOOD VESSELS	KIDNEYS	OTHER ORGANS OF UROGENITAL TRACT	LIVER	SPLEEN	LYMPH NODES	GASTROINTESTINAL TRACT	MISCELLANEOUS
17	E. L.	Zinc mining	10	8	Silico-tuberculosis	41	Silicosis, bilateral, all lobes Chronic pulmonary tuberculosis, bilateral, all lobes Emphysema, right lower lobe Chronic passive congestion	Chronic adhesive pleurisy, bilateral	Cardiac hypertrophy with dilatation	Acute nephritis		Hypertrophic cirrhosis Fatty degeneration		Chronic lymphadenitis, abdominal lymph nodes	Dilatation of stomach Chronic passive congestion, stomach and small intestine	
18	J. R. P.	Metal mining	13	11	Silico-tuberculosis	56	Silicosis, bilateral Chronic pulmonary tuberculosis with cavitation, right and left upper lobes and caseation, right and left lower lobes	Chronic adhesive pleurisy, bilateral	Cardiac hypertrophy with dilatation Fibrosis of myocardium Arteriosclerosis of aorta			Chronic hepatitis	Chronic splenitis Tuberculosis of spleen Chronic passive congestion	Chronic lymphadenitis, pancreatic lymph nodes	Dilatation of stomach	
19	J. J. D.	Anthracite coal mining	10	10	Silico-tuberculosis	41	Silicosis, bilateral Chronic pulmonary tuberculosis with cavitation, upper portion, left lower lobe Emphysema, right and left upper lobes and right middle lobe	Chronic adhesive pleurisy, bilateral	Cardiac dilatation, right heart Chronic myocarditis Fibrinous pericarditis				Chronic splenitis			

							middle lobe									
20	J. G.	Stone cutting	32	Unknown	Silicotuberculosis	47	Silicosis, bilateral Chronic pulmonary tuberculosis, right and left upper lobes Emphysema, right and left lower lobes Chronic passive congestion, right and left lower lobes	Chronic adhesive pleurisy, bilateral	Cardiac hypertrophy with dilatation, right heart	Chronic passive congestion		Hypertrophic cirrhosis				
21	J. J. R.	Granite cutting	Unknown	Unknown	Silicotuberculosis	59	Silicosis, bilateral Chronic pulmonary tuberculosis, with cavitation, right and left upper and left lower lobes Chronic interstitial pneumonia Emphysema, right lower lobe	Chronic adhesive pleurisy, bilateral	Cardiac dilatation, right heart	Chronic passive congestion			Chronic splenitis Chronic passive congestion		Tuberculosis, small intestine	Tuberculosis of adrenal glands Abdominal ascites
22	H. G.	Lead and silver mining	Unknown	2	Silicotuberculosis	47	Silicosis, bilateral Chronic pulmonary tuberculosis, bilateral, all lobes	Chronic adhesive pleurisy, bilateral Hydrothorax, bilateral	Cardiac hypertrophy with dilatation	Chronic passive congestion Chronic nephritis		Chronic passive congestion	Chronic passive congestion			

ASBESTOSIS

Asbestosis results from the inhalation of asbestos fibers over a period of years; usually a minimum period of seven years is required for a subject to develop the disease.

Chemically, the asbestos of commerce is a hydrated magnesium silicate consisting primarily of silica (combined silica) 44.1 per cent, magnesia 45 per cent, and water 12.9 per cent, while ferrous iron and nickel are present in small quantities.

Asbestosis is caused by a slow growth of fibrous tissue around the bronchioles, the smaller air tubes, and between the air cells. The principal sites for the deposition of asbestos are the bronchioles. While new fibrous tissue is being laid down, that deposited earlier gradually contracts and strangles the essential tissues of the lungs, both parenchymal and vascular. Following this, there is irritation of the bronchioles, which causes cough and an interference with the respiratory function, so that there is an impediment to the interchange of oxygen and carbon dioxide, with the result that a state of anoxemia develops, accompanied by dyspnea and hyperpnea.

Termination of the disease results either from a supervening infection such as lobar pneumonia, bronchopneumonia, or subacute tuberculosis, or, in the absence of an infection, cardiac hypertrophy and dilatation may ensue and cause death. The cardiac disease results from the widespread fibrosis and compression and obliteration of the capillary blood vessels of the lungs.

The gross pathological findings in the uncomplicated asbestosis lung consist of bands of gray fibrous tissue situated beneath the pleura with prolongations into the deeper portions. The interlobar septa are thick, and careful examination reveals deposits of slightly pigmented fibrous tissue along terminal bronchi that happen to be cut longitudinally. Cross sections of these structures appear as ill-defined areas of fibrosis. The lymph nodes at the root of the lung are not appreciably involved.

Histologically the changes in asbestosis consist of a chronic diffuse interstitial fibrosis of the lungs with areas of acute catarrhal inflammation. The characteristic asbestosis bodies may be seen either singly or in clumps. They are golden yellow in color, due to their high iron content and are formed by the development of an haustrated deposit upon the surface of the mineral fiber.

The present study includes but one case of asbestosis. The history of the case, together with the autopsy findings, follows:

F. G., a young white man, was discharged from the U. S. Navy in June, 1922, with no disability. He worked in the carding room of an asbestos plant for eight years. He began to complain of dyspnea, chest pain, cough, and fatigue and lost eighteen pounds in weight.

He was admitted to a Veterans' Administration Facility in April, 1930, at which time he complained of dyspnea, nervousness, and tremor of the hands

and appeared undernourished and ill. Sputum examinations were negative for the tubercle bacillus. His temperature was 37°C. with a pulse of 100.

Physical examination revealed the presence of bronchial breathing and medium râles over both upper lobes. A friction rub was also heard. He developed gastric distress, more dyspnea and cyanosis and died suddenly June 8, 1930, aged twenty-seven.

The autopsy findings were: Acute lobar pneumonia; asbestosis characterized by interstitial fibrosis; bilateral chronic adhesive pleurisy; cardiac hypertrophy and dilatation; and chronic appendicitis.

Microscopic study of sections of the lung showed the presence of a nonnodular fibrosis without any hyalinization. There was evidence of interstitial reaction from the pneumonia. In many places the air spaces contained pneumonic exudate composed of mononuclear and a few polynuclear leukocytes.

In the fibrous tissue and in the exudate were a great number of asbestosis bodies of all shapes and sizes. Some were free and others were inside of giant cells. The bronchi contained exudate. The capillaries and larger vessels were engorged and there was some blood inside of the air spaces.

DISCUSSION

The pathological findings in the autopsy material of this group of twenty-three cases indicate that silicosis is not only accompanied by characteristic fibrotic lesions of the lungs, pleurae, and lymph nodes, but may be complicated by tuberculosis or other pathological changes in the respiratory tract and in various organs.

It is noted that fourteen of the twenty-three cases showed the presence of a coexisting tuberculous disease. Tuberculosis as a complication of silicosis has been given intensive study by many investigators. It is a clinical observation that tuberculosis behaves atypically in the presence of silicosis. It is usually chronic in its course, frequently without any evidence of intoxication, and may exist for a long time without impairment of the working capacity of the silicotic. It may be endogenous or exogenous in origin.

The inception of clinical tuberculosis in the silicotic is dependent upon a number of factors such as the dosage of tubercle bacilli, and the state of immunity of the silicotic subject. If the number of tubercle bacilli is great, or if the immunity of the host is below normal, the tuberculous infection spreads and becomes manifest as clinical tuberculosis.

In the silicotic there are conditions favoring the multiplication of the tubercle bacilli and also factors which result in a lowered immunity of the host. The toxic action of silica on the lung parenchyma with necrosis of the silicotic nodules as well as the fibrotic changes in the lymphoid tissue furnish soil which is favorable for the growth and multiplication of the tubercle bacilli.

In addition, impairment of the lymphatic drainage which is the result of the extensive fibrosis, blocks the lymph flow so that there is interference with the ability of the lymph to eliminate tubercle bacilli. Accordingly, the organisms remain in the lymphoid tissue as well as in the lung parenchyma and multiply,—thus resulting in a spread of the tuberculous infection and in its clinical manifestation.

There is another way in which tuberculosis may enter the respiratory tract and develop in the silicotic:—by the exogenous route. This is a type of massive infection which enters the lungs and cannot readily be disposed of by the lymph eliminative mechanism or by the immunological response of the host. Under such circumstances the tuberculous infection spreads and assumes clinical importance.

The heart may also show evidence of disease,—disease which is not due to senescent degenerative changes, but to a slow and progressive dilatation and hypertrophy caused by increased effort due to obstruction of the pulmonary circulation, the result of extensive fibrosis of the lungs. The cardiac failure is insidious and slow and brings about a train of symptoms and pathological conditions such as dyspnea, chronic passive congestion of various organs, ascites, hydrothorax, etc.

The question may be asked whether or not the degenerative changes of the heart and of other organs, such as the liver and spleen, may be due in part to the toxic action of silica. If silica is toxic and produces pathological changes in the lungs, pleurae, and glands, why may it not produce similar changes in other organs? The answer to the question will require additional study of the cytological and histopathological changes produced by silica in the various organs and tissues, confirmed by similar observations in animals. As a matter of fact such suggestive

toxic action has been noted in experimental animals following the injection of silica particles into the circulation.

SUMMARY

The following is a summary of the principal pathological findings in the lungs and other organs of the twenty-three cases included in this study.

1. All twenty-three cases gave evidence of involvement of the pleurae. Chronic adhesive pleurisy was the usual finding. Involvement of the pleura may be the cause of the chest pain which is invariably present in silicotics.

2. Of twenty-three cases autopsied, fourteen gave evidence of an associated tuberculosis. In eight the two conditions were discernible as separate and distinct diseases; in six it was not possible to ascertain a separate background for either disease so that the combined condition was classified as silico-tuberculosis.

3. Tuberculosis complicated the incipient as well as the advanced stages of silicosis. Extra-pulmonary tuberculosis was a frequent accompaniment. It was miliary in type, having its inception in the pulmonary tuberculous disease.

4. A study of the location of the tuberculous disease of the lungs revealed that in five cases all lobes were affected; in two the disease was present in the right and left upper lobes; both lower lobes were affected in one; the left lower lobe was affected in one; and in five the distribution of the tuberculous disease was in one or both upper lobes as well as in the right middle or in one of the lower lobes.

5. Emphysema was not seen as frequently as was expected,—only eight of the twenty-three cases gave evidence of this condition; four of the eight were found in silico-tuberculosis; two in third degree silicosis and tuberculosis; one in first degree silicosis and tuberculosis; and one in first degree silicosis.

6. Chronic lymphadenitis was found in eleven of the twenty-three cases; the tracheobronchial lymph nodes were most frequently affected (nine cases).

7. In fourteen cases disease of the liver was present; three of the fourteen showed evidence of hypertrophic cirrhosis.

8. In twelve cases disease of the spleen was present,—six showing chronic splenitis.

9. The cardiovascular system was a frequent site of disease. Hypertrophy or dilatation of the heart, or a combination of the two conditions, was a common finding. Thus in three cases cardiac hypertrophy was present; in six cardiac dilatation was found; and in nine cases cardiac hypertrophy and dilatation were combined. In other words, eighteen of the twenty-three autopsied cases gave evidence of hypertrophy or dilatation of the heart, or a combination of hypertrophy and dilatation. The age incidence in the group of eighteen cases was from twenty-seven to seventy-two years; the average age was forty-seven and four-tenths years.

10. According to the autopsy findings in the eighteen cases, cardiac hypertrophy, cardiac dilatation, or a combination of the two conditions, was found in the early as well as in the advanced stages of silicosis.

11. Another finding was that cardiac hypertrophy, dilatation, or a combination of the two conditions, was found in silicosis complicated by tuberculosis.

12. Arteriosclerosis of the aorta was found in nine of the twenty-three cases; in five of these there was concomitant cardiac hypertrophy or dilatation. The age incidence of these nine cases ranged from thirty-seven to seventy-two years; three were in the fifth decade of life. The average age of the group of nine cases was fifty-two and eight-tenths years.

13. Chronic passive congestion of various organs due to cardiac failure was found in fourteen of the twenty-three cases.

The present study was made with the coöperation of a number of the medical officers of the Veterans' Administration. Coöperation in this study was given by Drs. Leroy U. Gardner and H. L. Sampson of Saranac Lake, New York, and Dr. Harry B. Williams of Veterans' Administration, Sunmount, New York. Technical assistance was rendered by E. K. Stone and A. Bambery, of the Research Subdivision.

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THE SIGNIFICANCE OF MUCUS IN CARCINOMA OF THE PROSTATE GLAND

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Although occasional cases of gelatinous carcinoma of the prostate gland have been observed, the frequent occurrence of mucus in smaller quantities in cases of prostatic carcinoma has not been recognized. In routine examinations of sections of carcinoma of the prostate gland at the clinic occasional areas were noted which had the appearance of mucus. It was therefore decided to stain sections from a considerable number of glands in which carcinomas were growing with stains specific for mucus and from them determine the frequency of mucus and its significance in this type of neoplasm.

In the literature and standard textbooks there is little information on this subject. Generally in the textbooks on histology there is no note concerning mucus in the epithelium of the prostate gland or in its secretions. Occasionally its occurrence is denied. Stieve, however, made the statement that the prostatic secretion stains weakly with mucicarmine. Apparently the only careful study of the prostate in regard to the presence of mucous glands and the formation of mucus is that of Schlachta who reported mucus-producing cells of two types, those which occurred occasionally in some of the prostatic acini, and those which occurred in true mucous glands, both types being more common along the ducts. Mucus-producing cells occur principally near the apex of the prostate gland distal to the utricle and in that portion of the gland anterior to the urethra. These regions of the gland are less often examined in routine histologic studies. Mucus-producing cells are most common in the fetus; they rapidly be-

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come less numerous after birth until, in adults, they are very few in number and difficult to find. Concerning prostatic carcinoma, Simmonds said that colloid degeneration could occur. Oberndorfer thought that colloid carcinoma of this organ belonged among the most extreme rarities. Apparently Boyd reported the first case of colloid or mucoid carcinoma of the prostate gland. His diagnosis was made on gross examination without the aid of any special staining technic. More recently, Klissurow made a careful detailed study in another case of colloid carcinoma.

METHODS

In this study sections were made from tissue removed during transurethral resection in seventy-one cases of adenocarcinoma, in four cases of squamous-cell carcinoma, and in thirteen cases of ordinary benign hyperplasia of the prostate gland. Generally, three to five pieces of tissue were examined in each case. Sections were also made from normal glands obtained at necropsy in ten cases, the subjects having varied in age from being stillborn to eighty-three years. These glands weighed less than 15 gm. and microscopically were within the limits of normal. All the specimens had been fixed in 10 per cent formalin for periods of from two months to three years. Sections from each case were stained with hematoxylin and eosin by a standard technic, and with mucicarmine according to the following technic: (1) The formalin-fixed tissue was embedded in paraffin, (2) sections were cut at 8 microns, (3) sections were then deparaffinized, (4) sections were placed for twenty minutes in picric acid solution, which was made by adding 5 cc. of glacial acetic acid to 75 cc. of a saturated aqueous solution of picric acid, (5) sections were then washed fifteen minutes or longer in running tap water, and (6) stained in alum hematoxylin for five minutes, (7) they were again washed well in tap water, (8) immersed in a saturated aqueous solution of lithium carbonate for one minute, (9) washed in tap water, (10) stained in mucicarmine for twenty minutes, (11) washed in water, (12) dehydrated in 95 per cent alcohol, acetone, carbol xylol, and (13) mounted in balsam. The mucicarmine stain was made fresh each time by mixing 4 grams of carmine (Alum Lake), 1 gram of aluminum chloride, and 8 cc. of water. This was heated slowly for several minutes and then made up to 400 cc. with 50 per cent alcohol. Mucicarmine stains mucus and tissue containing mucus a bright red, in contrast to the surrounding tissue which is stained pale blue.

Each carcinoma was graded microscopically according to Broders' method for determining the degree of malignancy. A method was then devised for grading the amount of mucus in the sections, although this was difficult because of its irregular distribution. If any mucus at all was present in any portion of any section, the case was classed as being of grade 1 for mucus. If a fourth

to a half of the acini or cell groups of any given microscopic field in any portion of the tissue contained mucus, then it was classed as grade 2 for mucus. When more than half the cell groups contained mucus it was classed as grade 3, and those cases were considered as being of grade 4 for mucus when any microscopic field was found to contain mucus in more than three-fourths of its area.

Not all of the microscopic fields showed the same amount of mucus. Occasionally in a section classed as containing mucus of grade 4 there would be microscopic fields containing none at all. Generally the number of areas containing mucus was proportional to the mucous content of the area showing the maximal amount of mucus. In a section classed as grade 4 because of a very large amount of mucus in one particular microscopic field there would generally be mucus in nearly every microscopic field and, usually, much mucus in every field. On the other hand, a section classed as grade 1 because of the relatively small amount of mucus in a given area would reveal only a few areas containing mucus while most of the fields contained none at all.

RESULTS

In the sections of ten normal prostate glands mucus was found in four. There was only the slightest trace of mucus in each of these four cases and, in two of them, the mucus was definitely in a short gland adjacent to the urethra and not in the deeper acini of the prostate gland itself. Table 1 and figure 1 illustrate the distribution of mucus in the other cases: There were thirteen cases of hyperplasia of the gland. Some mucus was present in every case, nine of them having mucus of grade 1, two mucus of grade 2, and two mucus of grade 3. There were eight cases of grade 1 adenocarcinoma of the prostate; one with mucus of grade 1, three with mucus of grade 2, two with mucus of grade 3, and two with mucus of grade 4. Of twenty cases of grade 2 adenocarcinoma of the prostate gland, two showed an absence of mucus, twelve mucus of grade 1, two mucus of grade 2, three mucus of grade 3, and one mucus of grade 4. Out of twenty-six grade 3 adenocarcinomas of the prostate, ten revealed no mucus, nine mucus of grade 1, four mucus of grade 2, two mucus of grade 3, and one mucus of grade 4. In the seventeen grade 4 adenocarcinomas of the prostate, there was no mucus in eleven, four contained mucus of grade 1, one mucus of grade 2, and one mucus of grade 3. There was no mucus in any of the four cases of squamous-cell carcinoma of the prostate gland.

TABLE 1

AMOUNT OF MUCUS PRESENT IN PROSTATE GLAND

PROSTATE GLAND	TOTAL CASES	NO MUCUS		AMOUNT OF MUCUS							
				Grade 1		Grade 2		Grade 3		Grade 4	
		Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent
Normal	10	8	80	2	20	0	0	0	0	0	0
Hyperplasia	13	0	0	9	70	2	15	2	15	0	0
Adenocarcinoma, grade 1	8	0	0	1	12.5	3	37.5	2	25	2	25
Adenocarcinoma, grade 2	20	2	10	12	60	2	10	3	15	1	5
Adenocarcinoma, grade 3	26	10	39	9	34	4	15	2	8	1	4
Adenocarcinoma, grade 4	17	11	65	4	23	1	6	1	6	0	0
Squamous cell carcinoma	4	4	100	0	0	0	0	0	0	0	0

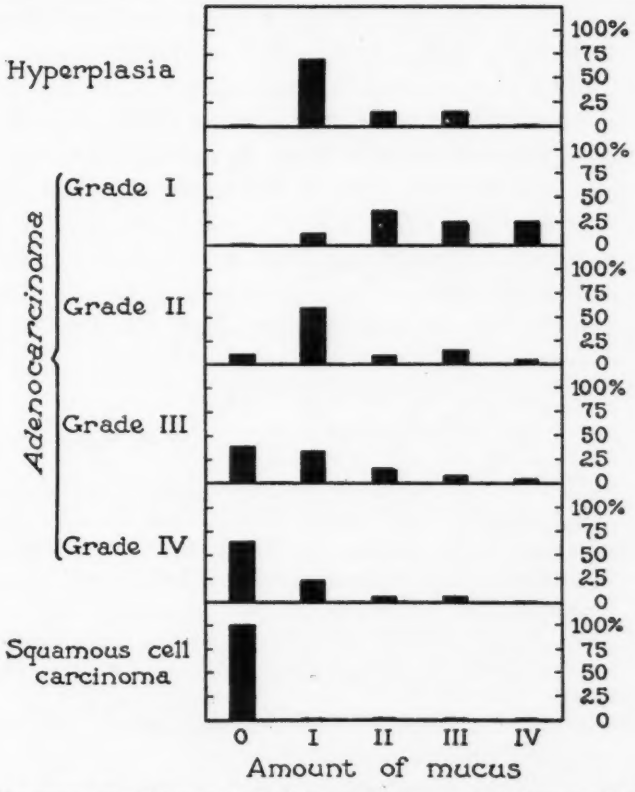

FIG. 1. SHOWING THE AMOUNT OF MUCUS PRESENT IN VARIOUS LESIONS OF PROSTATE GLAND

COMMENT

It is at once apparent that the amount of mucus present in prostatic carcinomas is definitely related to the degree of malignancy. Its amount is inversely proportional to the degree of malignancy, the lower the degree of malignancy the greater being the production of mucus. On the other hand, when the degree of malignancy was high, the production of mucus was small. Even the high-grade carcinomas contained mucus only in their somewhat slower-growing areas, the extremely rapidly growing areas containing no mucus whatever.

Embryologically the prostate gland is derived from the urethra. It is the most highly specialized gland derived from this source and has a specific function. Ordinarily the adult gland contains very few mucous cells and produces little mucus. Other glands derived from the urethra, which are less highly specialized, have as their principal function the production of mucus. Schlachta observed considerable mucus in the young developing prostate of the fetus. The occurrence of mucus in carcinoma of the prostate gland may be due to the less differentiated and less specialized carcinoma cell reverting to a more primitive function of mucus formation which is rarely exhibited in the fully differentiated adult cell of the prostate. In the more rapidly growing high-grade carcinomas, the cells are too undifferentiated to function as producers of mucus.

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EDITORIAL

The Ideal Vaccine*

The specificity of vaccines and the efficacy of vaccine therapy have been subjects for study since even before the days of Pasteur. Jenner's remarkable success with cowpox vaccine, later rationalized by the early studies in bacteriology marks the first of many attempts to prevent or treat infectious disease according to basic immunologic principles. Some have been successful, many have failed. In some instances failure has been due to the use of the wrong organism, as in pertussis and influenza, while in many more results have been poor even though the actual etiologic agent was employed. Although there is much of which we are still ignorant, we have reached a stage where we now understand more clearly the desiderata for an ideal vaccine.

The recognition and separation of the virus diseases has been a distinct forward step. Assuming that present belief is correct, that influenza, acute coryza, poliomyelitis, epidemic encephalitis, measles, varicella, etc. are virus infections, there will be no more fruitless attempts at prevention with bacterial vaccines. The recognition that virus diseases may be prevented only with living virus has stimulated research in their attenuation which we may hope will eventually be productive. The development of virus vaccines is a field in and of itself.

Bacterial vaccines are in quite another category. Early successful work, as with typhoid and cholera vaccination, brought out that repeated inoculation with increasing strength is often more successful than single injections. This is a step forward which however was found not to solve all vaccination problems.

Prophylactic vaccination against tubercle bacillus infection remains a puzzle. Calmette and Guerin, possibly borrowing from observations on virus infection as well as from the fact that

*Received for publication December 23, 1937.

lowgrade tuberculous infection appears to protect against reinfection, have carried on extensive investigations in the use of an attenuated living organism. This remains in the experimental stage. Immunologic studies of the tubercle bacillus and of other bacteria, especially the pneumococcus have given us what appears to be a clearer understanding of the nature of bacterial allergy and its possible relationship to bacterial immunity. The present status may be briefly summarized as follows.

Although Rosenau and Anderson demonstrated very early that guinea pigs may be sensitized to tubercle germ substance and may be made to react anaphylactically thereto, the clinical evidence of sensitization is the familiar delayed or tuberculin type reaction. Tubercle germ substance may be divided into a nucleoprotein fraction, apparently responsible for the tuberculin type reaction, and a soluble residue. Pneumococcus has likewise been divided into a group-specific protein and a type-specific carbohydrate. The latter appears to act as a hapten. The presumption of bacteriologists is that hapten also exists in tubercle germ substance but is destroyed during the process of fractionation. It is not likely to be destroyed in those microorganisms which are more easily fractioned, by autolysis, such as the pneumococcus.

Although bacterial haptens like other haptens cannot sensitize except when combined with the specific protein, they can produce allergic shock, positive skin reactions of the early wheal type and positive reactions on passive transfer by the Prausnitz-Kustner technique.

Those who are interested in bacterial allergy report that the characteristic positive reaction to any of the common respiratory vaccines is of the delayed tuberculin type but that occasionally one observes early wheal type reactions. The suggestion seems appropriate that in the absence of a wheal reaction to a bacterial vaccine, the probability is that the organism has been so denaturized that its presumptive hapten component has been destroyed. If this should turn out to be the case it is at once obvious that such vaccines are not ideal because they have been too greatly denaturized.

One possible reason for failure in some conditions has been the difficulty of making a vaccine or bacterial extract in which the test substance may be said to be absolutely identical to the organism which was removed from its focus in the patient. In the production of bacterial anaphylaxis animals were sensitized with bacterial substance which had been grown artificially and were shocked following reinjection of the identical material. This is quite a different process from that of obtaining the presumptive bacterial antigen from a source of infection in the body, growing it on artificial culture media, and killing it either with heat or chemicals. The test material can scarcely be considered identical with the original. Sterilization by grinding in a ball mill until each individual bacterium is disrupted should prevent the denaturization that presumably accompanies heat or chemical treatment, but even so, the character of the culture medium may theoretically at least cause some alteration in antigenicity. It has been found for example that typhoid vaccine grown on a protein rich medium is more likely to produce chill and fever when given intravenously than is one which was prepared on a protein poor medium. The change in antigenic capacity may be both quantitative and qualitative. There is evidence for example in food allergy, that the nutritive source may cause sufficient alteration to remove allergenic activity. Thus Kern has described a patient who has symptoms after eating Michigan celery but can eat celery grown in Pennsylvania with impunity. The present writer has a patient who can eat Colorado celery but not Michigan celery. This same person can eat California oranges but not Florida oranges. Another patient cannot eat California oranges but has no symptoms from Florida oranges.

It would appear, therefore, that the most nearly ideal vaccine should be one which is sterilized in a ball mill and which is grown on a medium which contains human blood as the chief nutritive ingredient. Undoubtedly many other modifications in the technique of vaccine preparation will be necessary before the ideal vaccines for all organisms have been prepared.

W. T. VAUGHAN.

NEWS AND NOTICES

The Registry of Medical Technologists broke all records in the number of applicants for the last semi-annual examinations. Of the 568 original entries, there were 69 cancellations on account of illness or other reasons. Of the balance 464 passed the examination successfully and 35 failed. The practical and written tests were held in various parts of the United States and Canada under the direction of 124 clinical pathologists. The total number of Registrants now runs up to the sizable figure of 4467.

Many inquiries reach the Registry from certificate holders who seek post graduate instruction in a particular field of laboratory work. Data are now being secured and a directory assembled where facilities for such studies are available. In this connection a summer course in parasitology is announced by the Rocky Mountain Biological Laboratory, at Gothic, Gunnison, Colorado, combining a delightful environment in the heart of the Rocky Mountains with instruction by experts in this branch and at very moderate cost. Those interested may write for details to Dr. John C. Johnson, 26 Price Street, West Chester, Pennsylvania.

The first regional seminar in hematology was held in Rochester, Minnesota, on February 25 and 26, 1938. There were twenty men in attendance from the States of North Dakota, Minnesota, Wisconsin, Iowa, Indiana and Illinois. Two sessions were held each day, from 9:00 to 12:00 a.m. and from 2:00 to 5:00 P.M.

On the first morning, Dr. Byron Hall discussed the histogenesis of blood cells. This was followed by a resumé of the morphologic findings as well as some clinical data on the anemias by Dr. Frank J. Heck. The rest of the morning was devoted to a study of blood smears of the anemias.

The first afternoon, Dr. Malcolm Hargraves talked on "Toxic infectious changes in the blood and the blood picture in infectious mononucleosis."

The second morning, Dr. Charles Watkins discussed the morphology of the leukemias, and in the afternoon Dr. Hal Downey, of the University of Minnesota, talked on "Reticulo-endotheliosis."

It seems desirable, in seminars of this type, to present in conjunction with the morphologic discussion a brief resumé of the salient facts as regards the symptomatology as well as treatment. At the present time, collections of slides are being made of the various blood dyscrasias so that this material will be available to other groups throughout the country who might wish to conduct similar seminars.

There is available to the membership of the Society now, a set of blood slides of the commoner blood dyscrasias which can be obtained from the Hematologic Registry under the following conditions:

1. A deposit of $5.00 will be required to cover the slides. Upon return of slides, $3.00 will be returned to the member, the rest being retained to cover cost of shipping and expense in maintaining this service.
2. The slides may be kept a maximum of sixty days. If they are kept beyond this time the member will be charged rental at the rate of $1.00 for each thirty days or fraction thereof.

Slides may be obtained by addressing Dr. Frank J. Heck, Mayo Clinic, Rochester, Minnesota.

In response to a call issued by the New York State Counselors of the American Society of Clinical Pathologists fifty-five pathologists met at dinner in Albany on February 26, 1938 to discuss the desirability of forming a state association. Following the election of temporary officers it was voted to organize such a society, and a committee of nine was elected to draw up a constitution. The next meeting will be held in New York City at the time of the Annual meeting of the Medical Society of the State of New York in May when a permanent organization will be effected.

"Dr. Benjamin" and "Dr. Moran"

In the March Journal attention was called to a specialist in rubber checks calling himself "Dr. Benjamin."

This man, or a similar individual recently appeared in Spokane, Washington as "Dr. Moran," but was unsuccessful in his endeavor to cash a check. In order that he may not succeed elsewhere his description is again published together with added information forwarded from Spokane by Dr. Robert F. E. Stier.

The man is of average size, about 40 years old with hair tinged with gray. His face is round with small, dark, slightly prominent eyes. He has a short, thick neck. The Spokane adventurer showed an oval scar, apparently from an operation to uncover the parietal bone, which he states to have been a decompression for a fractured skull following an automobile accident. About an inch above the ear there is a depression.

Both of these men—if they are two—talk fluently about pathology and pathologists.

Information of the arrest of one of these men has recently been received.

On to San Francisco

Present indications suggest that the coming Convention in San Francisco June 9, 10, and 11 will be replete with interest.

The Palace Hotel will be the headquarters of the Society. All reservations are being handled by Dr. Frederich C. Warnshuis, 450 Sutter Street, San Francisco.

The Seminar this year will be devoted to a study of "The Pathology of The Skin" and will be conducted by Dr. Lee McCarthy of Washington, D. C. in coöperation with Col. J. E. Ash of the Army Medical Museum.

BOOK REVIEWS

Diseases of the skin, for Students and Practitioners. By OLIVER S. ORMSBY, M.D., Clinical Professor and Chairman of The Department of Dermatology, Rush Medical College of The University of Chicago, Dermatologist to the Presbyterian and St. Anthony's Hospitals, Chicago, etc. Revision of The Histopathology and Mycology by CLARK WYLIE FINNERUD, B.S., M.D., Assistant Clinical Professor of Dermatology, Rush Medical College of The University of Chicago, Assistant Attending Dermatologist, Presbyterian Hospital, Chicago. Cloth. 5th Ed. 1334 pp.; 658 illustrations and 3 colored plates. $12.00. Lea and Febiger, Philadelphia, Pa.

Those already familiar with this excellent book will not be surprised that it has reached a fifth edition. Those hitherto unfamiliar with it will recognize at once that it is an outstanding comprehensive and authoritative presentation well worthy of the reputation it has attained.

The practical experience of the author is apparent on every page. He writes well and clearly and very obviously has a remarkably comprehensive understanding of his subject.

The present edition has added descriptions of twenty new diseases and forty-four new illustrations and the entire text has been subjected to extensive revision.

The difficulty of forming clear cut pictures of skin affections from description alone has long been apparent. The numerous illustrations in this book are not only well chosen but excellently reproduced. The book will, therefore, prove of great use and value, not only to the student and physician, but also to the pathologist who will also be especially interested in the discussion of the histopathology and mycology of the diseases of the skin.

All in all, this book can be recommended without reserve as a well-planned, well written and eminently practical and valuable text.

Clinical and Experimental Investigations in Agranulocytosis, with Special Reference to the Etiology. By PREBEN PLUM, Nyt Nordisk Forlag Paper. 410 pp., 26 colored microphotographs and numerous other figures. H. K. Lewis Co., Ltd. London.

This is one of the most comprehensive studies of agranulocytosis this reviewer has encountered, embracing not only a thorough study of the literature, but detailed accounts of 114 cases personally studied by the author, 36 of which came to autopsy.

The extent of this study can be seen from the table of contents: Historical;

Occurrence; Clinical Symptoms; Hematological Signs; Laboratory Examinations; Etiology; Pathogenesis; Diagnosis; Prognosis; Course; Treatment.

From his own studies, as well as by analysis of the work of other investigators, the author is convinced that the most frequent cause of agranulocytosis is amidopyrine and emphasizes the fact that the presence of this drug may be concealed by a variety of proprietary names.

In view of the frequency and importance of this condition this study is a contribution of outstanding value and importance.

Lectures on the Epidemiology and Control of Syphilis, Tuberculosis, and Whooping Cough, and Other Aspects of Infectious Disease. By THORVALD MADSEN, M.D., Director of the State Serum Institute of Denmark, Copenhagen, Chairman of The Health Committee, League of Nations. Cloth. 216 pp.; 72 figures. $3. Williams & Wilkins Co., Baltimore.

This book contains the fifth series of Abraham Flexner Lectures given in the School of Vanderbilt University. This volume, though small, contains a wealth of information.

The first lecture contains a comprehensive and authoritative account of the measures developed in Denmark for the control of venereal disease with special reference to syphilis.

In view of the present interest in this problem in the United States, this lecture is of great interest. It is of especial interest to note that the campaign in Denmark had its beginning as long ago as 1776 and underwent many vicissitudes prior to the law of 1906 under which the program is now carried out.

The second lecture discusses in detail the mechanism of bacterial infection; the third, tuberculosis; the fourth, the influence of seasons on infection; and the final lecture, discusses whooping cough.

These lectures are of great interest and value and this book well deserves a place on the bookshelf of every physician.

Approved Laboratory Technic: Clinical Pathological, Bacteriological, Mycological, Parasitological, Serological, Biochemical, and Histological. By JOHN A. KOLMER, M.D., DR.P.H., SC.D., L.L.D., L.H.D., F.A.C.P., Professor of Medicine, Temple University; Director, Research Institute of Cutaneous Medicine; Formerly Professor of Pathology and Bacteriology, Graduate School of Medicine, University of Pennsylvania; and FRED BOERNER V.M.D., Assistant Professor of Bacteriology, School of Medicine and Graduate School of Medicine, University of Pennsylvania; Bacteriologist, Graduate Hospital, Philadelphia. Cloth. Ed. 2. 893 pp.; 12 colored plates and 380 text illustrations. D. Appleton-Century Co., Philadelphia.

This second edition, rewritten, extensively revised and reset is indubitably a better and more complete text than its predecessor. Not only has the entire book been subjected to a thorough revision, but new chapters have been added covering Methods for The Hormonal Diagnosis of Pregnancy, Hydatidiform

Mole, Chorionepithelioma and Teratoma of The Testes, Diagnostic Mycological Methods, Methods of Examination of The Skin and Mucous Membranes for Animal Parasites, and Histological Methods and The Preparation of Museum Specimens. The sections on Parasitology and Toxicology have been thoroughly revised and improved.

The twenty-eight contributors are all well known and thoroughly competent in their respective fields and their discussions may be regarded as authoritative.

Without doubt, this volume will continue as before a standard and valuable reference text.

Manual of the Diseases of the Eye, for Students and General Practitioners. By CHARLES H. MAY, M.D., Consulting Ophthalmologist to Bellevue, Mt. Sinai and French Hospitals, New York; Formerly Chief of Clinic and Instructor In Ophthalmology, Medical Department of Columbia University and Director of The Eye Service at Bellevue Hospital, New York. Cloth. Ed. 15. 376 illustrations, 78 colored figures. William Wood & Co., Baltimore.

This book is so well known as a standard text in numerous medical schools that it is only necessary to say of this fifteenth edition that it fully duplicates the exceptional standard of its predecessors.

Not So Long Ago. A Chronicle of Medicine and Doctors in Colonial Philadelphia. By CECIL K. DRINKER, M.D., Sc.D., Professor of Physiology and Dean of The School of Public Health, Harvard University. Cloth. 183 pp. $3.50. Oxford University Press, New York.

This is a book of such intriguing interest that, once begun, it is difficult to relinquish until the last page has been turned—with regret.

Based upon the diaries kept by the author's great-great grandmother during the years 1758–1807 it presents a vivid and unforgettable picture of life in Colonial Philadelphia and, particularly, of medical practice in Colonial times.

In these modern days it is impossible, without the help of such records as these, to appreciate the total lack of sanitation, the primitive bathing facilities, and the accepted habits of life of even well-to-do families which were, after all, as the title of the book suggests, "not so long ago."

It is, however, very easy to appreciate how inevitable were the plagues of malaria, dysentery, typhoid and yellow fever common in those times; all of which are graphically described in homely language. Fortunate in their circumstances, the family had for medical attendants such historic figures as Benjamin Rush, William Shippen, Philip Syng Physic, Adam Kuhn and John Bard who appear in the pages of the diary as living personalities.

While the diary of Elizabeth Drinker has become an historical document and has been utilized before in part in recording the history of Philadelphia during the Revolution, it has not hitherto been used to present the picture of daily life in Colonial Philadelphia.

Dr. Drinker has chosen with exceptional skill such excerpts as form a balanced and connected story, not only of life from day to day, but one presenting a vivid picture of Colonial medicine and Colonial doctors.

There is so much in this book of varied and continued interest that one is tempted to regret the absence of an index for ready reference. For this is a book to be read and reread with increasing pleasure.

It is difficult to imagine any one to whom this book would not prove of absorbing interest.

Surgical Pathology of the Diseases of the Neck. By ARTHUR E. HERTZLER, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kansas, Professor of Surgery, University of Kansas. Cloth. 237 pp.; 206 illustrations. J. B. Lippincott Co., Philadelphia.

The book maintains the high standard of the previous monographs on Surgical Pathology by the same author.

Its outstanding characteristic is its obvious foundation in experience, not only extensive but digested and analyzed with understanding. Professor Hertzler speaks from personal knowledge and extensive study, and speaks without ambiguity.

The thoroughness with which the subject is covered is seen by the table of contents: Preview of The Surgical Affections of The Neck; Hodgkin's Granuloma (Lymphoblastoma); Lymphosarcoma; Lympho-endothelioma; Rare Primary Tumors of The Neck; Diseases of Vestigial Rests; Benign Tumors of The Neck; Diseases of The Salivary Glands; Secondary Tumors of The Neck; Inflammatory Affections of The Neck.

This is a book which well deserves a place in the library of surgeon, pathologist, and physician and which can be read not only with profit but with pleasure.

The numerous illustrations are not only excellent but excellently reproduced. No mistake will be made in the purchase of this volume.

A Handbook of Accepted Remedies. Edited by P. J. HANZLIK, M.D. Paper. Ed. 2. 115 pp. $1. Issued by the Department of Public Health, San Francisco, J. C. Geiger, Director. Published by J. W. Stacey, Inc., San Francisco.

This is a small pocket-size hand book containing much of interest to the interne and, indeed, to the physician at large.

It contains, in addition to 57 pages listing drugs, their doses, indications and contraindications, brief summaries of the treatment of emergencies, symptoms and treatment of poisoning, diagnostic procedures, and miscellaneous information.

Tumors of Bone (Including the Jaws and Joints). By CHARLES F. GESCHICKTER M.D. and MURRAY M. COPELAND, M.D. With Forewords by Dean Lewis,

M.D. and the late Joseph Colt Bloodgood, M.D. Cloth. Revised Edition. 832 pp., 525 figures. The American Journal of Cancer, New York.

This revised edition of a well known and authoritative text upon tumors of the bone will be cordially welcomed by surgeon, pathologist, roentgenologist and physician alike.

The foreword by Dean Lewis presents in succinct and striking manner the interpretation of clinical findings, while that by the late Dr. Bloodgood presents the rules of diagnostic and therapeutic procedure for bone lesions.

The main text has been extensively revised to include the newer data relative to tumors related to osteogenesis, a rewriting of the sections devoted to Ewing's sarcoma and the osseous changes produced by lymphoma and blood dyscrasias; and the addition of chapters on tumors of the cranial bones, the jaws and the tendon sheaths, joints and bursae. The supplement dealing with bone diseases has also been enlarged.

The volume is profusely illustrated and, as the case with publications from this source, are not only excellent in character but superbly reproduced.

All in all this is an excellent and valuable book of especial interest to the pathologist.

Cutaneous Cancer and Precancer. A Practical Monograph. By GEORGE H. MACKEE M.D., Professor of Clinical Dermatology and Syphilology and Director of The Skin and Cancer Unit, New York Postgraduate Medical School and Hospital, Columbia University, and ANTHONY C. CIPALLARO, M.D., Associate in Dermatology, Skin and Cancer Unit, New York Postgraduate Medical School and Hospital, Columbia University. With a Foreword by FRANCIS CARTER WOOD, M.D. Cloth. 222 pp., 245 illustrations. The American Journal of Cancer, New York.

This is, indeed, a practical book, useful to the practitioner at large as well as to the specialist. Not offered as a reference book it is clearly, as described in the Preface, a manual based upon undisputed facts and experience.

The main sections of the book discuss: I, The Morbidity and Mortality of Cutaneous Cancer; II, The Precancerous Dermatoses; III, Carcinoma and Sarcoma; and IV, Established Therapeutic Methods. In each section the symptomatology, diagnosis, etiology, pathology and treatment of precancer and cancer of the skin and mucosa are discussed clearly, succinctly and intelligibly.

The numerous illustrations are of outstanding excellence. Each chapter concludes with a pertinent bibliography and an index makes the contents readily accessible.

This is a book the pathologist will be glad to have and the physician should enthusiastically welcome.

Early Medieval Medicine, With Special Reference to France and Chartres. By LOREN C. MACKINNEY PH.D., Professor of Medieval History, University of

North Carolina. Cloth. 247 pp., 9 plates, $2.75. Johns Hopkins Press, Baltimore.

In this volume are presented the Noguchi lectures delivered by Professor MacKinney before the Johns Hopkins Institute of The History of Medicine.

The volume is divided into three main sections: I, The Dark Age Concept and Early Medieval Medicine; II, Medicine in Merovingian and Carolingian France; and III, Medical Progress at Chartres in the Tenth and Eleventh Centuries.

To those interested in medical history—as all physicians should be—this little book will prove of absorbing interest. Professor MacKinney has woven in well chosen words a panoramic tapestry depicting medicine in the Middle Ages based upon extensive research and original documents as shown in the fifty-eight pages of notes appended to the text.

The medical historian will receive this volume with pleasure and enthusiasm. To the reader at large, and to the physician who may have neglected this phase of his chosen profession, this book will present a story of absorbing interest.

This volume may be recommended with enthusiasm and without reserve.

Fever Therapy. Abstracts and Discussions of Papers Presented at The First International Conference on Fever Therapy. Edited by WALTER M. SIMPSON, M.D., and WILLIAM BIERMAN, M.D. Cloth. Pp. 486, $5.00. Paul B. Hoeber, Inc., New York.

There is little doubt that fever therapy represents an epoch-making therapeutic advance. Still in its infancy, this subject has awakened international interest and this book, therefore, presenting abstracts of the papers read at the First International Conference on Fever Therapy contains much of great interest. Both the abstracts and the discussions of the papers are presented in English, French and German.

While it is to be regretted that space did not permit the publication of the papers in full, the abstracts present a clear survey of the varied and important phases of this new type of therapy which seems to possess an ever-increasing field of usefulness.

ERRATA

VOLUME 8, MAY 1938, NUMBER 3, PAGES 255–264

Article by Drs. F. H. Lamb and R. L. Jackson

Legend	under	Figure	5	belongs	under	Figure	7
"	"	"	6	"	"	"	8
"	"	"	7	"	"	"	9
"	"	"	8	"	"	"	10
"	"	"	9	"	"	"	5
"	"	"	10	"	"	"	6

CLINICAL PATHOLOGY TODAY*

CARL W. MAYNARD

"Apart from the guarantee of our own convictions, the observable direction of living nature is our guarantee of right." (Julian Huxley)

Custom decrees that each president of this society shall render an up-to-date report on the state of the clinical pathologist, as he understands it. My predecessors have done their duty in this respect with noteworthy success. They have defined the clinical pathologist as "a consulting physician whose chief interest lies in the diagnosis of disease by laboratory methods." They have shown that the clinical pathologist is an inevitable and essential product in the evolution of scientific medicine. They have recognized the fact that since his contacts are chiefly with fellow physicians rather than with patients, he must be of exceptional personality and training. They have demonstrated that both the patient and the patient's medical attendant benefit from his counsel. Indeed, in the diagnosis of malignant disease, and in the development of hospital organizations, he has been classed as indispensable.

The economic phase of the specialty is influenced by numerous factors, among which may be noted the facts that much medical laboratory work is institutional; that technical diagnostic procedures can be carried out by highly trained workers who are not physicians; and that current practice in medical education presumes that all graduates will possess a certain degree of skill in the use and interpretation of laboratory findings. Based on these accepted facts it has been suggested that clinical pathology is a specialty only of convenience, not of necessity. A survey of the practice of medicine today establishes beyond question

*Presidential Address before the 17th Annual Convention of the American Society of Clinical Pathologists, San Francisco, June 9–11, 1938.

the essential importance of the specialty. Unless the future brings lesser ideals and lower standards of scientific excellence in the care of the sick, the necessity for physicians specially skilled in diagnosis by laboratory methods will continue.

When the first clinical pathologist began his practice he was pioneering—a missionary with a new medical gospel. Today every medical student has the opportunity to learn the elements of laboratory diagnosis. The graduating interne enters practice more or less familiar with the common laboratory procedures, and for a time may perform them himself. He can employ a technician, and frequently does so. If his technician has a current certificate from the Board of Registry, she should do reliable work. This is a proper procedure, and the only way many unhospitalized patients can be furnished prompt and economical routine laboratory assistance. But the basic sciences are extensive and changing. The general physician cannot keep up with them as his clientele increases. Within a few years his medical school technique is forgotten or superseded, and he does need, and call for, assistance from the laboratory specialist.

The clinical pathologist is a physician, not an overpaid technician. He developes technical procedures for the benefit of the sick, then plans for their widest possible use. Measures which are universally known, easily applicable, and self-interpreting are properly carried out by each physician for his own patients. The rhinologist does not treat all cases of rhinitis, nor the ophthalmologist every conjunctivitis. In the assembling and development of useful laboratory aids, in the training of technicians and the supervision of their work, and in the field of tissue pathology, the clinical pathologist is established. His leadership in a most progressive phase of medical diagnosis will continue to be indispensable.

As to the present condition of the clinical pathologist, in June, 1938, there are, however, several phases worthy of notice.

1. The prevalent opinion that laboratory costs are high, if not excessive. Every reader is familiar with the current expression "expensive laboratory service." This idea was prominent in the report of the Committee on the Costs of Medical

Care, and in that report was linked with the suggestion that laboratory services should be rendered by the State or by its subdivisions. A recent commentor suggests that the pathologist has been less vocal in keeping his work before the profession because so much of his work is mechanical. The failure of the Committee on the Costs of Medical Care so much as to mention the Clinical Pathologist as a part of the medical picture, was undoubtedly due to ignorance on the part of the Committee, and ignorance could well have been due to the lack of a pathologist spokesman. We have been too much devoted to mechanical work with not enough real practice of clinical pathology.

Certainly laboratory tests do cost something; even more certainly their direction and interpretation must be considered as part of the costs of medical care; and just as certainly does this expense contribute as much to the patients' welfare, dollar for dollar, as does any other part of the healing art. Consultation fees need not be charged for the simplest of routine tests, and charges are adapted to low incomes by every physician. The expression "expensive laboratory service" should be replaced by "indispensable laboratory service."

2. The variety of situations in which the clinical pathologist is carrying on his activity, and the opportunities in these situations.

The American Society of Clinical Pathologists has 533 members, which includes approximately half of the physicians who are eligible for membership. A rather loose classification indicates that of this number there are about 27 teachers and research workers, 265 hospital pathologists, 44 group clinic pathologists, 168 pathologists practicing privately, and 29 public health workers; federal, state, county and municipal.

The intimate correlation of teaching and research prevents more than a formal division under the two terms. In fact many research problems are receiving competent attention from workers in each of the subgroups of clinical pathology.

The teacher must fit the medical student with a fundamental understanding of the laboratory sciences in their relation to the diagnosis and treatment of disease. He may well be ex-

pected to present the need for the specialist in clinical pathology, his professional advantages, and what he may expect financially. Contemporary attempts by members of the medical profession, and others, to promote governmental aid in financing medical schools may prove to be an early step in changes which will involve the clinical pathologist as a teacher, along with the rest of the educational organization.

Pure research endeavors likewise are subject to economic influences. Endowments rise and fall, commercial possibilities stimulate current financing, and the federal government contributes. Opportunity is here for the pathologist who is qualified by ability and training.

The field of the hospital laboratory is by nature a large one, and has its own problems, often discussed before this Society. The concentration of patients necessitates a concentration of diagnostic facilities. Since a certain part of hospital laboratory work is routine, a repetition of well-established procedures, it is proper that we have secured technical assistants to carry out this routine. Large institutions, well endowed, furnish abundant opportunity for the clinical pathologist as organizer, director, and consultant. The smallest hospitals, dependent entirely upon earnings, must have such technical help as they can afford, and too, such professional laboratory supervision as they can afford. The hospital neither large nor small presents a special problem. Here earned income can furnish equipment and technicians, but the struggling institution often accepts the clinical pathologist as a too expensive technician rather than as a valuable member of the staff. As a compromise, the combination pathologist-roentgenologist has been an economic success in some institutions. Hospitals furnish concentrated opportunity and concentrated difficulty. From the standpoint of service to the sick, it is certainly in the hospital that the pathologist-consultant finds his greatest opportunity.

The furnishing of laboratory service to the ambulatory patient and to those confined in homes, is as large a field as that of the hospital, but scattered over much more territory. It is here that the private clinical pathologist is particularly needed. In

his absence, and for routine work under the supervision of a physican employer, the technician is of definite value, but there is a natural, though unfortunate, tendency for the employer to ask more than routine services from his technician. Here the clinical pathologist can be of assistance by teaching, in medical society and hospital staff meetings, the use and interpretation of measures which the clinician and his technical assistant can safely employ. Such educational activity is a valuable part of the practice of clinical pathology, and may also remind the medical profession that consultation from the laboratory is available in diagnostic problems.

In the group clinic laboratory is found another plan for giving to the nonhospitalized sick diagnostic consultation as well as routine laboratory service. Each community has its own particular conditions which determine whether the group clinic or the private laboratory will best meet the needs of the profession. The cooperative laboratory, with pathologist and technicians furnishing unlimited but properly chosen diagnostic aids to the patients of the physicians associated, has been found satisfactory in some locations.

Three branches of the federal government provide opportunities for a limited number of experienced laboratory workers.

The Navy employs pathologists in all naval hospitals and on board the hospital ships. These pathologists are commissioned officers in the Navy.

In the Army there are 49 officers who are listed as laboratory officers and pathologists. One has the rank of Colonel, 29 are Lieutenant Colonels, five Majors, and fourteen Captains. Work done under their direction is similar to that in the laboratory of the general hospital. Some of the laboratories also do water and food analysis.

The Public Health Service has in its professional ranks eight men who are classified as pathologists. In addition to this the Service includes 38 clinical medical technicians and 41 research medical technicians. Except for three technicians at the National Institute of Health, the technical assistants of the Public Health Service are understood to be under immediate professional supervision.

State, county and municipal health departments should present abundant openings to physicians trained in clinical pathology. Valuable work in preventive medicine is done by the tax-supported laboratories of the United States. Where appointments are permanent, subject to Civil Service, attractive work is available for competent clinical pathologists. There are tendencies, however, in public health laboratory work which keep it from harmonizing fully with medical ideals. Political influence has been known to supply incompetent technicians to directors who wish only expert assistance. Again, the ambitious laboratory chief may do all the work his public health field calls for, then seeking valid argument for increased support, be led to expand beyond the poorly-defined limit between preventive laboratory service and the practice of general clinical pathology. This tendency, though difficult to defend, is natural, and is part of the background of changing ideas in medical practice.

3. Much of the agitation for increased governmental activity in the care of the sick is being based upon the idea that there is a social class, the low-income group, neither indigent nor affluent, which cannot easily pay for medical care, and which accordingly is chronically suffering from lack of proper attention. Regardless of the merits of the agitation, the subject has received much publicity. The apparent need for a new type of medical practice has thus been urged, first by the Committee on the Costs of Medical Care, and more recently by the American Foundation, which has assembled and published portions of letters from selected physicians, under the title "American Medicine." The evidence selected for emphasis from the testimony given, and the occasional forced conclusion, suggest that these investigations have been planned as propaganda for the socialization of medical practice. Whatever the purpose, a portion of the press has acclaimed the material presented as newsworthy.

A considerable number of county medical societies have established plans for helping the low-income group in the financing of adequate medical care. This has been done by physicians who have seen a definite way to be of service in today's social economy. By physician-controlled prepayment or postpayment

contracts, individuals and families having incomes between $900. and $1800. a year, or thereabouts, are being given the privilege of choosing a doctor from the medical society's members, and receiving personal care, payment being adjusted in some way to fit within the available budget. Just as group clinics have succeeded partly in somewhat similar work, these experiments by medical societies are partly successful, and with experience should become of great value, if the need for them continues to exist. The clinical pathologist should fit easily into this picture. If the method involves the adjustment of charges by every cooperating physician, the pathologist is of course included. If payment is prorated from a prepaid fund, laboratory advice will be called for with less hesitation than has often been the case under purely individual practice. If hospitalization is included in a prepayment plan, the pathologist's services should be paid for, with those of roentgenologist and anesthetist, as professional care, not as part of the hospital per diem. Each community has to work out appropriate details in its own medical society plan, and the pathologists in each group must join in deciding how medical care is best to be furnished.

4. The possible eventuality of a nationwide insurance project, federally controlled and tax supported, furnishing medical care and hospitalization to the entire populace, cannot be ignored. If it comes, the clinical pathologist should fit into the plan as well as others with special fields of work. The organization of considerable numbers of physicians, under whatever auspices, creates an increased opportunity and demand for physicians of special ability and training. We will do well, however, to learn from the experience of clinical pathologists in England. According to Dr. S. C. Duke, President of the Association of Clinical Pathologists, the British State Medical Service provides no facilities whatever for laboratory aids to diagnosis. After the Association was organized about eleven years ago, an attempt was made to have laboratory investigations included under the panel benefit. For a time success seemed possible, but it has never been attained. Efficiency demands, but other government expense prevents. The voluntary hospitals in some cases allow

the use of their laboratory facilities by panel physicians, and so in part help make up for the deficiency of the State Medical Service.

State Medicine may not become a reality in the United States soon, but the clinical pathologists should have a share in whatever planning is being done.

To summarize the situation as reviewed:

1. Clinical pathology has evolved as a necessary part of medical practice.

2. Technical assistants can legitimately perform routine laboratory tests for clinician employers who are prepared to make their own interpretations and assume responsibility therefor.

3. The clinical pathologist is entitled to adequate professional compensation for diagnostic consultation, for laboratory supervision, and for other professional services.

4. The American Society of Clinical Pathologists includes in its membership several types of workers, the majority of whom supervise hospital laboratory services, or combine private laboratory practice with hospital work.

5. A small number of clinical pathologists are found in the various government services.

6. Clinical pathologists will be essential in health insurance plans equally with other specialists, whether the plans are controlled by the medical profession or by the federal government.

7. The American Society of Clinical Pathologists should share in the planning of improved medical care for the low income group.

ELLIPTICAL ERYTHROCYTES IN HUMAN BLOOD*

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Aside from the poikilocytosis of anemias, the erythrocytes of man exhibit such remarkable constancy of shape that individuals showing oval and elliptical erythrocytes rather than the normal circular biconcave discs interest the observer. With the exception of the terrestrial family, *Camelidae*, the erythrocytes of all mammals are circular. Conversely, amongst the lower vertebrates the cells are elliptical with the single exception of the marine family, *Cyclostomata*.

Dresbach in 1902 observed the first recorded case of elliptical erythrocytes in man and to date, 75 authentic and 25 doubtful cases have been reported in the literature. The majority have been seen in normal healthy persons without an anemia, although attention has been drawn to the person or family by hospitalization of a member for another malady. Associated conditions have ranged from mere malaise to malignancy and treatment varied from symptomatic medication to splenectomy, none of which have affected the phenomenon. Anemia present was usually accounted for by the concurrent disease. Males and females are equally affected. The age varied from 82 years to 11 months.[11,19] It is not confined to those of negro blood or ancestry as is sickle cell anemia,† but has been observed in Americans of Dutch, Italian, Scotch Irish, Spanish, Jewish, and Russian Jewish, and Negro descent from divergent portions of

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† There are a few reports in the literature of sickle cell anemia in white persons but since the Moorish invasion of Spain and the former prevalance of slavery in Europe and America, it is perhaps difficult to prove that a given individual does not have some negro blood in his distant ancestry.

the country. It has been observed also in native Germans, Austrians, Italians, Dutch and Austrian mulattos.

The familial tendency of the phenomenon was suspected by Dresbach, suggested by the cases of Bishop and of Huck and Bigelow, and firmly established by Hunter and Adams in 1929. Their Dutch family of 18 members embracing three generations was further studied by Van den Bergh in the Holland branches. Five genealogical charts have been recorded including Cheney's family showing 14 members with elliptical erythrocytes over a span of four generations. The phenomenon appears to be a non sex-linked Mendelian dominant character. Direct transmission is common; indirect has not been proven. Whether it must be in the immediate parent to be transmitted is still controversial.[9,16,18] Blood grouping throws no light on the ethnology of the anomaly as all groups are represented in cases although Group I Jansky is the least frequently represented.

The etiology is unknown. It has been laid at the door of a faulty erythropoiesis, influences of the blood plasma, the inherent quality of the cell, racial characteristics and anoxemia and it has been suggested that sickle cell anemia has a similar etiology with this phenomenon. A permanent transmission cannot be affected by transfusion[6] and normal erythrocytes are unaltered by the plasma of persons with the anomaly.[12,13,16,19] Autopsies,[16] bone marrow studies[9,16,17] and splenic punctures[9,13,15,17] evidence the fact that the cells are not elliptical as they are formed in the bone marrow but assume that shape when subjected to the influence of some unknown constituent of the plasma or extra-marrow tissues. That normal erythrocytes are unaffected by the plasma from a case suggests a second intraerythrocytic susceptibility to the extraerythrocytic influence, either or both of which may be congenital. Such a conception would, of course, color the Mendelian pattern in the f^1 and f^2 generations, a fact noted by McCarty.

Most observers have found that mechanical effects (faulty technique),[8,10,11,16,19] temperature,[17,19] 24 hour hanging saline drop preparations[5,6,9,12,16,17,19,20] normal blood serum,[6,8,12,14,16,17,19] single isotonic saline washing,[6,8,9,12,14,17,19] hypotonic saline

washing,[11,14,17,19,8] picric acid,[20] carbon dioxide,[20,13] nitrous oxide,[20] oxygen,[16,20] cholesterol,[19] anticoagulants,[14,16,19] and the appearance of a state of anemia[11,14,19,20] all failed to affect the constancy of the ellipsoid state of the erythrocytes. Since there is generally a greater percentage of elliptical cells in the wet preparations than the dry smears, mechanical effect is hardly a feasible cause. It is significant that neither carbon dioxide nor oxygen affect the number of elliptical erythrocytes; though it is known that carbon dioxide decreases the percentage of sickle cells in a wet preparation and that the number of sickle cells increase in a wet preparation on standing. No such increase in the wet preparation is noted with elliptical cells.

Pollock and Dameshek found that at 5°C. there was a definite decrease in the number of oval cells compared to the number at room temperature (24°C.) and at incubator temperature (37.5°C.) Hunter and Adams claim that all the cells (elliptical) became discoid in shape at 54°C. Repeated washings in isotonic saline caused a change of the ellipsoid cells to round forms, a fact not observed in the poikilocytes of anemia.[16,17]

That elliptical erythrocytes are more resistant to hemolytic agents and have a decreased fragility has been pointed out by most observers, especially in the fine work by Terry. In hypotonic solution, Terry and Bernhardt have shown that some of the ellipsoid cells become round before hemolysis occurs, a finding not observed in the poikilocytes of anemia. These findings with repeated washing with isotonic saline and with hypotonic saline suggest that the elliptical erythrocyte is not a true poikilocyte but an anomaly. Bleeding time, clotting time, clot retraction, platelets, icteric index and Van den Bergh test are all found to be normal. Terry, ingeniously, has shown that the elliptical cells are heavier than the round cells. Stephens and Tatelbaum in an excellent study, found that there was a decrease in the mean corpuscular volume and hemoglobin content involving both the round and the elliptical cells of persons exhibiting the phenomenon. Pollock and Dameshek observed that potassium cyanide increased the number of elliptical erythrocytes very rapidly. Lecithin was found by McCarty to produce a change of the ellipti-

cal cells to round cells, and Kanellis has produced an artificial poikilocytosis in normal blood with the same substance. In no case has there been 100 per cent elliptical erythrocytes in the blood.

The anomaly is to be distinguished from sickle cell anemia by the absence of crescent shaped erythrocytes, although elliptical cells, transitional forms, are to be seen associated with the true crescent cells of sickle cell anemia. Also, in elliptical cells, there is no increase in the number of abnormal cells on standing in wet preparation and no decrease in CO_2 mixtures, as is always observed in sickle cell anemias.[16, 17, 19]

Huck and Bigelow found that it took two months for elliptical erythrocytes of a donor to disappear from the normal circulating blood of a recipient (case six). This observation, properly evaluated in the light of a suggested increased resistance to hemolysis, may be of some significance in the consideration of the life duration of the erythrocyte in vivo.

Moreover, a medicolegal significance, heretofore ignored, may be attached to the rather constant congenital characteristic of of the erythrocyte which is not associated with disease or altered by therapy. Within certain limits it may be used as a means of identification of both person and paternity.

The rarity of the phenomenon is more apparent than actual. In series of non-selected cases, the incidence of elliptical erythrocytes in small numbers has varied from 0.04 to 3 per cent, the lower percentages probably being more correct.[19, 20]

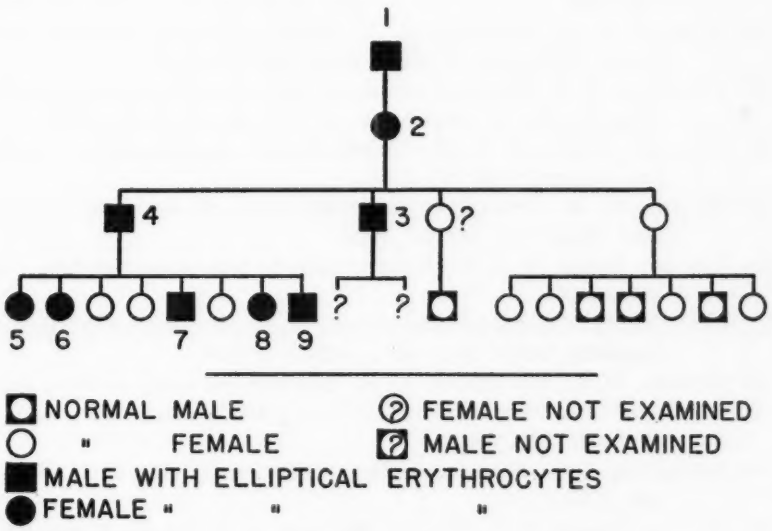
Case study. J. S., 84 year old white, German American male was initially admitted with prostatic hypertrophy. After operation the cystotomy wound failed to heal and he was treated intermittently for a period of six years during which his blood studies showed the following range: R.B.C., 3.25 to 3.64 million; Hemoglobin—55–70 per cent; Kahn negative; icteric index—0.6; secondary anemia.

Of 25 descendants in four generations, twenty were studied. However, cooperation was very poor and it was not possible to secure a dry smear from any but the patient. In addition, the family was suddenly scattered and lost by the Ohio valley flood of 1937. Of the twenty relatives studied, nine showed abnormal erythrocytes. Five males and four females were affected. Their

age ranged from 30 days to 84 years. Except for the above patient all affected members were in good health.

NUMBER	NAME	AGE	SEX	ABNORMAL ERYTHROCYTES	REMARKS
				per cent	
1	John S.	84	M	84*	1. Father of 2.
2	Ethel S. H.	60	F	60	2. Mother of 3, 4.
3	Charles H.	33	M	80	
4	John H.	30	M	60	4. Father of 5–9.
5	Violet H.	13	F	55	
6	Catherine H.	10	F	35	
7	Charles H.	6	M	40	
8	Rose H.	3	F	60	
9	Francis H.	30 days	M	44	

* Since this article was written there has appeared another family of three generations in which ten members exhibited elliptical erythrocytes. (Hereditary Ovalocytosis: Observations of ten cases in One Family, M. B. Strauss and G. A. Daland, N. E. Journ. Med., **217**: 100–104, 1937.



SUMMARY AND CONCLUSIONS

The presence of elliptical erythrocytes in the human blood is a familial phenomenon appearing as a non-sex-linked Mendelian

dominant characteristic which is not associated with any known disease and unaffected by any tried therapy. The etiology is unknown. Seventy-five authentic and twenty-five questionable cases have appeared in the literature and include five families, one of which affected four generations. Nine additional cases, in four generations, are reported. The elliptical erythrocyte is not a poikilocyte of any of the known anemias, including sickle cell anemia. It appears to be a congenital anomaly compatible with an otherwise normal healthy individual.

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LEUCOCYTOSIS ASSOCIATED WITH ACUTE INFLAMMATION*

ANDERSON NETTLESHIP†

But little is known of the mechanism which produces leucocytosis in acute inflammatory conditions. From the area of local damage some stimulus must travel to the bone marrow to cause its heightened activity.

Understanding of this phenomenon may be gained by studying acute inflammatory lesions in relation to the concurrent leucocytosis. In the present study small lesions were produced in the skin of the rabbit. These were studied to see if necrosis at the site of inflammation may be related to leucocytosis.

Experiments

The hemolytic streptococcus isolated by Gay[1] was the inflammatory agent. Rabbits with a normal blood picture were used. The usual blood techniques were employed, smears being stained with Wright's stain. Lateral ear vein blood was obtained, care being taken not to traumatize the ear as this produces a leucocytosis (Schattenberg[2]). The blood picture was studied every two or three hours during the first forty-eight hours, after that every twelve to twenty-four hours to termination of the experiment. Thirteen animals were injected into the skin of the back with 0.1 cc. of a suspension of a 1:100 dilution of a twenty-four hour broth culture of the virulent organisms. Six control animals were injected with the same quantity of broth—they remained normal throughout the experiment. Total averages, in thousands of leucocytes, of the thirteen which received the virulent organisms are given in table 1. Total time is two weeks.

Leucocytosis set in within an hour after injection. There was a rapid rise, the leucocytes increased 100 per cent above normal by the eighth day, one animal at that time having a leucocytosis of 50,000, and leucocytosis of 25 to 35 thousand was commonly seen. This is not clearly brought out by the table since each animal was at the height of its leucocytosis at a slightly different time

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† Fellow in Medicine, The National Research Council. (This work was begun in the Department of Pathology, Cornell Medical School.)

from the others. Peak heights persisted for eight to ten days. Though most animals showed a return to normal at three weeks some had a moderate leucocytosis at that time. Leucocytosis is brought about by an increase in polymorphonuclear cells. Young forms make up part of the increase after the first forty-eight hours.

In the skin, at the site of injection, a small reddened area, pointed with edema and a tiny center of necrosis, was often visible between the eighth and twelfth hour. This pustule became rapidly larger during the first twenty-four hours, the necrosis being as large as 10 by 20 millimeters at that time. Necrosis spread less rapidly during the next twenty-four hours. Chart 1 shows a typical case, animal number 1004. The gross appearance of the surface of the abscess can not always be correlated, in time and amount of necrosis, accurately with the leucocytosis since most of the necrosis is hidden beneath the skin surface. A very accurate correlation with the micro-section is possible.

TABLE 1

TIME AFTER INJECTION	LEUCOCYTES	TIME AFTER INJECTION	LEUCOCYTES	TIME AFTER INJECTION	LEUCOCYTES
	thousands		*thousands*		*thousands*
Before	9.0	48 hrs.	17.0	9th day	20.0
3 hrs.	13.7	3 days	21.1	10th day	24.3
6 hrs.	17.0	4th day	18.1	11th day	19.1
8 hrs.	13.7	5th day	20.0	12th day	24.1
12 hrs.	18.0	6th day	21.9	13th day	16.0
24 hrs.	16.1	7th day	22.4	14th day	19.0
36 hrs.	16.6	8th day	24.5		

Histologic changes following injection. The microscopic changes will be described chiefly from the changes taking place in the leucocytes. Haemotoxylin and eosin, Verhoeff van Gieson and eosin methylene blue stains were employed.

Two hours. At two hours the collagenous fibers are slightly spread apart by edema. Scattered groups of polymorphonuclears are already present, most abundant where there are masses of streptococci. Many leucocytes show cytoplasmolysis (fig. 1). (Cytoplasmolysis as used here and throughout designates cells whose cytoplasm is "fading out", or the leucocyte which retains a bare rim of cytoplasm, or simply naked nuclei.) Few leucocytes show nuclear damage. Blood stream leucocytosis is 25 per cent above normal.

Four hours. Edema is more advanced and the number of leucocytes somewhat greater (fig. 2). Leucocytosis is rising.

Eight hours. Edema is marked, leucocytes present in abundance, about five times as many as at two hours. Their granules are often swollen and many show marked cytoplasmolysis. Karyorrhexis is slight, only an occasional

nucleus shows more advanced lysis. Collagen fiber damage is slight, there being no demonstrable connective tissue or elastic fiber damage. All these structures are spread apart by edema but they retain their outlines and internal structures. Blood stream leucocytosis continues to rise.

Twelve hours. Collagen fibers show distinct disintegration. There is a small center of central necrosis, affecting in most part leucocytes. Leucocytoplasmolysis is extreme. Leucocyte nuclei, for the first time, show marked karyorrhexis and karyolysis. Leucocytes are infiltrating in enormous numbers.

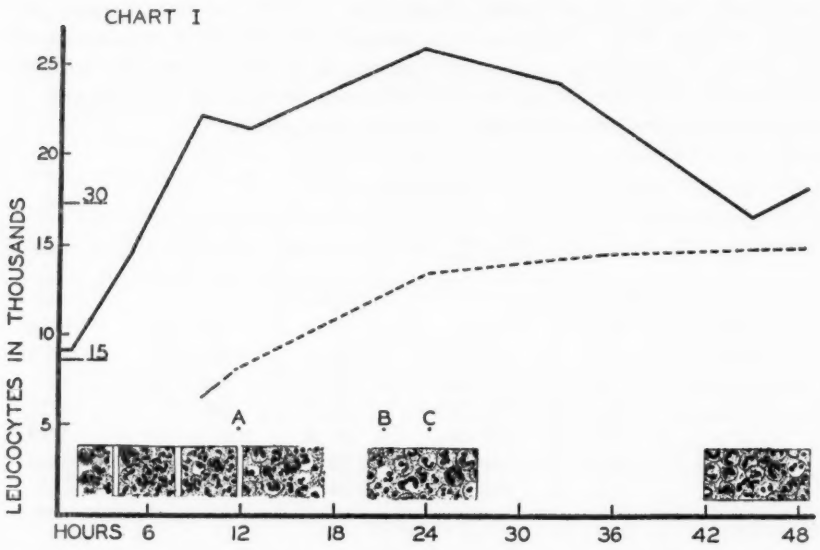

CHART 1. This chart illustrates the general blood stream leucocyte changes and local changes following injection of 0.1 cc. of a 1–100 dilution of a 24 hour broth culture of hemolytic streptococci. The heavy unbroken line represents the leucocytosis and is typical for the group. The light broken line is the area of visible necrosis (in the gross) given in square millimeters. The bottom pictures show the microscopic changes affecting the leucocytes in the abscess. Note early necrosis—2 hours. At point *A* the collagen fibers first show necrosis—12 hours. At *B* the connective tissue and elastic fibers are beginning to necrose. And at *C*, 24 hours, the epidermis and associated structures are beginning to necrose. Animal number 1004.

An occasional monocyte is seen about the outer edge of the lesion. Generalized leucocytosis is 100 per cent or above.

Twenty-four hours. Though nuclear degeneration is more marked than at twelve hours one is struck by the relative slightness of this in comparison to the

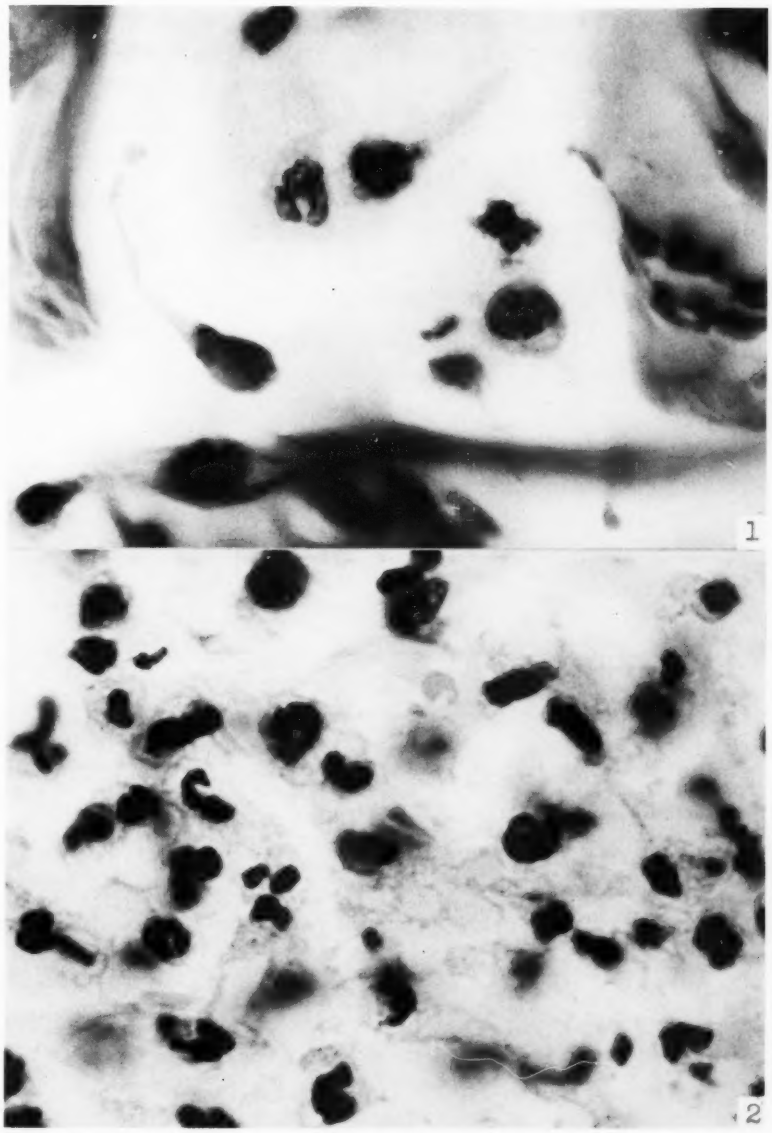

FIG. 1. Leucocytes in a two hour area of injury (0.1 cc. of a 1–100 dilution of broth culture). The amount of cytoplasmolysis is striking, there being but one intact cell visible in the field. ×1700.

FIG. 2. Leucocytes in a four hour area. There are usually eight to ten such groups to be found in the area of streptococcus injection at this time. Here, too, cytoplasmolysis is noted in a large per cent of the cells. ×1300.

amount of cytoplasmolysis which is extreme (approximately one-third of all leucocytes, many of which are completely stripped of their cytoplasm). There is marked swelling and fraying of the collagen fibers. Epidermis and associated structures stain poorly and are beginning to necrose. Leucocytosis remains over 100 per cent above normal.

Forty-eight hours. A piece of epidermis has sloughed out. Leucocytes are packed in the abscess tightly. There are a few fair sized hemorrhages. Blood stream leucocytosis is well over 100 per cent above normal, in some animals over 200 per cent.

Animals sacrificed at the second, fourth and tenth days showed marked hyperplasia of the bone marrow.

In summary: Streptococci introduced into the skin of the normal rabbit produced acute inflammation accompanied by an early, marked, prolonged leucocytosis. Cytoplasmolysis of leucocytes at the site of injection set in within two hours after injection and became more marked during the first twenty-four hours of inflammation, at which time the most rapid blood stream increase in leucocytes occurred. The cytoplasm of the leucocytes was the only tissue which necrosed early. Necrosis of the other tissues involved in the abscess did not occur to any extent until after twenty-four hours.

DISCUSSION

Leucocytosis of acute inflammation differs from that termed physiological leucocytosis (Garrey, W. E. and Bryan, W. R.[3]); there is, therefore, no need to discuss physiological leucocytosis here.

The mechanism which may be responsible for generalized leucocytosis with acute inflammation, investigated in this study is: that some breakdown product from the action of the injuring agent on peripheral tissue diffuses into the blood stream to cause leucocytosis and bone marrow hyperplasia.

The sections studied show that the tissues at the abscess site necrose at different times. In order of their necrosis were noted, leucocytes, collagen fibers, connective tissue and elastic fibers, epidermis and associated structures. The time of necrosis of these various structures is readily related to the general leuco-

cytosis. In the gross we have observed that as necrosis advances so advances leucocytosis. Microscopically, when the necrosis of each peripheral tissue at the site of injection is analyzed in relation to the time of leucocytosis, the one tissue whose damage comes shortly before and which necroses parallel to the blood stream leucocytosis is the white cells at the site of injection. Since necrosis of the nuclear material of the leucocytes occurs relatively late (18 to 24 hours) the observations point to the cytoplasm of the leucocytes as containing the "factor," which travels in the blood stream from the site of damage to produce myeloid hyperplasia and leucocytosis. Observations on human disease further the point. Lobar pneumonia causes a high leucocytosis in its early stages. The one tissue which shows marked necrosis early is the leucocytes in the alveoli.

Since His and Zinsser[4] first used leucocyte extracts in treatment of infections a great deal of work has been done on this. Thompson (quoted by Alexander[5]) first found that the injection of leucocyte extract gave a marked leucocytosis. This has been widely confirmed. The leucocytosis is primary and not preceded by a leucopenia. Why leucocyte extract causes a leucocytosis is made clear from the present work; the leucocyte extract acts on the bone marrow just as do the breakdown products (may be the same substance) from disintegrating leucocytes in the abscess.

A tremendous amount of work on protein and protein breakdown products, injections of all sorts, has been done. One of the more recent of such studies is that of Doan *et al.*[6] With such injections there is a primary leucopenia followed by a leucocytosis. The studies point to a primary leucocyte damage, the breakdown products resulting from the primary damage causing the leucocytosis.

Through study of the abscesses it was observed that there was a distinctly greater necrosis in the forty-eight hour abscess than in the twenty-four hour one. The advancing edge of necrosis is not increased proportionately. Because of this, and the fact that the center of the abscess is necrotic at forty-eight hours, the absorption of breakdown products is not so great. The leucocyte curve is beginning to flatten out. Another factor accounting for

the slowing down of acceleration may be that the bone marrow becomes increasingly difficult to stimulate.

Since leucocytes go to pieces continually in the blood stream, the mechanism for increased leucocyte production, suggested by the present experiments, may simply be an accentuation of the normal. The normal growth of bone marrow may be controlled by the usual breakdown products from the continually disintegrating leucocytes.

I am grateful to Doctor E. L. Opie for his help during this work.

SUMMARY

1. Acute inflammation caused by the injection of hemolytic streptococci intracutaneously is accompanied by a marked, prolonged leucocytosis. The leucocyte rise is made up of polymorphonuclear cells. There is marked bone marrow hyperplasia.

2. Necrosis of the cytoplasm of leucocytes infiltrating the injured area is closely connected with generalized leucocytosis. Necrosis of other damaged tissues is not directly temporally related to the leucocytosis.

3. Substances released from the cytoplasm of necrosing leucocytes appear to diffuse into the blood stream and cause the leucocytosis and bone marrow hyperplasia which accompany acute inflammation.

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FOCAL FATTY CHANGE OF LIVER AND FOCAL CIRRHOSIS*

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Unusual manifestations of fatty change of the liver and cirrhosis merit careful study, since many phases in the development of both these processes are still obscure. Both lesions are sometimes observed in association in the same liver and the former is common to a wide variety of clinical conditions. In contrast to the diffuse form, focal fatty change and focal cirrhosis are rare. Although the nature of these focal processes is not clearly understood, several examples of their spontaneous occurrence have been reported in medical and veterinary literature. Their rarity, however, in human pathology is curiously contrasted with their frequent occurrence among certain domestic animals as the horse, dog, sheep and, particularly, cattle. These peculiar localized lipoidal deposits have been variously designated "focal fatty infiltration of liver," "fatty infarcts" and "lipoma of liver." Whatever may be their pathogenesis, the term "infarct" is obviously a misnomer in this connection for many, sometimes all the cells within the area of involvement appear viable, the nuclei frequently appearing essentially normal. No evidence exists at present for regarding the process as a lipoma, a lesion which is apparently without precedence as occurring in the human liver.

In this communication two new examples of focal fatty change of the liver in man are presented together with a review of the literature, discussion of etiology and consideration of the possible relationship to cirrhosis.

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† Deceased.

Case reports

Case 1. E. S., white female aged 30 years, entered Jefferson Hospital with an abdominal fecal fistula of 2 years' standing and marked secondary anemia. The fistula followed appendectomy 2 years previously and had resisted 2 earlier attempts at repair. Shortly after admission the patient was again subjected to laparotomy, the fistula closed and an ileocolostomy performed. Continuous vomiting developed postoperatively and within 2 days the tract reopened. At a fourth operation a month later the terminal ileum and cecum were excised but circulatory collapse developed and the patient died 6 hours later.

Post mortem examination. (15 hours after death.) The combined gross and microscopic diagnoses were: (1) focal fatty change of liver; (2) surgical ileocolostomy and resection of intestine for post appendiceal fecal fistula; (3) chronic ulcerative colitis with polyp formation and diverticulum; (4) spontaneous fistula of transverse colon; (5) bilateral chronic suppurative salpingitis; (6) chronic fibrinopurulent peritonitis with extensive adhesions; (7) abdominal hemorrhage; (8) acute degeneration of myocardium, kidneys and liver; (9) solitary chronic granulomatous lesions in lymph node and liver.

The important findings were in the abdomen and pelvis. A recent abdominal incision enclosed a gauze drain which entered the peritoneal cavity. Extensive fibrous adhesions were attached to the anterior and inferior surfaces of the liver. A large amount of extravasated blood lay behind the ascending colon with small clots on both surfaces of the liver and throughout the abdomen. Several patches of purulent exudate adhered to the lower abdominal and pelvic peritoneal surfaces. The cecum and appendix were absent and the terminal ileum and beginning colon freshly ligated. An old ileocolostomy was functioning at the mid-portion of the transverse colon and the proximal colon had been closed at a previous operation. Thus, the ascending and half the transverse colon were tightly ligated at both ends and formed a closed sac except for a spontaneous fistula which is described below. The ileum beyond the ileocolostomy was patent for a distance of 28 cm. Its terminal end was found tightly ligated. The mucous membrane of this terminal segment of the ileum was congested and edematous and the contents consisted of opaque granular blood-stained fluid. The cecal end of the colon was narrow, its wall thick and fibrous and the mucous membrane ulcerated with 2 small polyps and a diverticulum. The transverse colon, immediately proximal to the ileocolostomy, contained the opening of a spontaneous fistulous tract which coursed posteriorly and terminated retroperitoneally in a large mass of chronic granulation tissue. Pus filled both fallopian tubes which were thickened, kinked and firmly bound by adhesions to the uterus, ovaries, pelvic wall and intestines.

The liver weighed 1190 grams. A dark brown patchy mottling and dense adhesions somewhat obscured the normal liver markings. There was a bright yellow area in the duodenal impression extending from about the level of the inferior margin to the tip of the caudate process (fig. 1). This area was roughly

quadrilateral, measuring 6 x 3 x 1.5 cm. A smaller, more deeply situated area measured 1 cm. in diameter. These areas were uniformly bright yellow, sharply demarcated from the surrounding parenchyma and although the liver markings were indistinct, there was practically no change in consistency. The gallbladder and bile ducts were patent.

Microscopic examination. Sections of liver tissue selected from regions distant to the localized yellow areas showed degeneration and necrosis of about 30 per cent of hepatic cells and signs of regeneration in many of the remainder. No mitotic figures were observed. A few hepatic cells about portal radicles were vacuolated. There was slight proliferation of small bile ducts and moderate increase in portal connective tissue which was infiltrated with small round cells, mononuclears and occasionally polymorphonuclear leukocytes and eosinophiles. An unusually large number of inflammatory cells were found in the

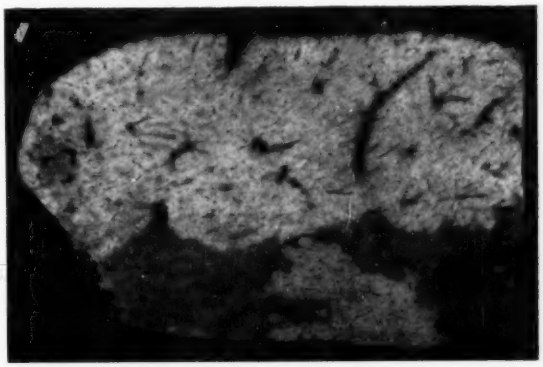

FIG. 1. Case 1. Cut surface of caudate process of liver through large area of fatty deposit near surface and smaller one more deeply situated.

sinusoids together with several small areas of focal necrosis in the parenchyma. One section contained a single chronic granulomatous lesion resembling a tubercle. At several places the liver capsule showed fibrosis and chronic inflammatory changes. In the sections stained for fat a small number of large lipoid droplets were observed in the hepatic cells at the periphery of the lobule. The Kupfer cells were entirely free of lipoidal material.

Examination of the fatty area confirmed the sharply circumscribed appearance noted grossly. The line of demarcation passed through various portions of the lobules and portal areas. In comparison with the surrounding tissue, the fibrosis and chronic inflammatory reaction in the portal areas appeared more marked. All the hepatic cells were vacuolated except a few at the portal areas and a narrow border immediately beneath the liver capsule. These vacuoles were of a uniformly large size and signet ring forms of the hepatic cells were the

rule. Degenerative changes in the cytoplasm were difficult to detect owing to extensive vacuolization but regressive nuclear changes were no more marked than in the non-fatty liver. With fat stains the lipoid droplets were uniformly large, sometimes crescentic, vacuolated in appearance and distributed throughout the lobule as noted above. Throughout both the fatty area and the remainder of the liver, neutral fat was demonstrated by the Nile blue sulphate, scarlet red and osmic acid stains. In preparations stained by the 2 former methods almost all the droplets were intimately associated with unstained needle-shaped crystalline material which produced marked irregularity in the

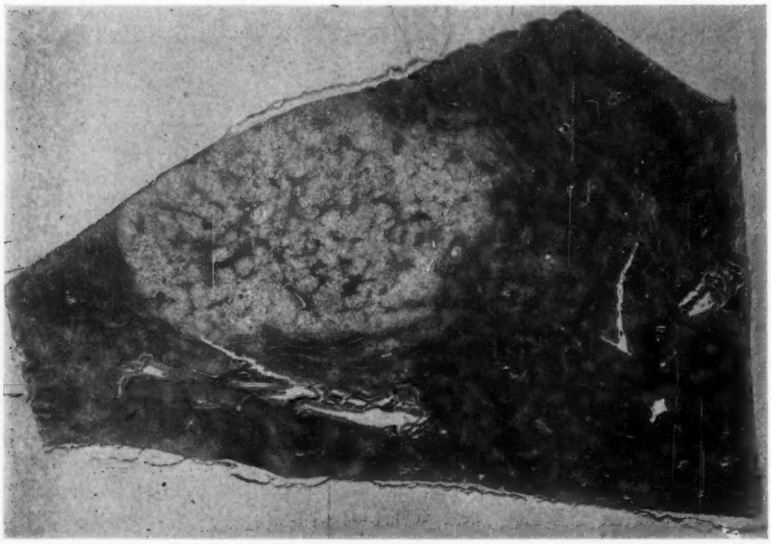

FIG. 2. Case 2. Section of liver containing pale rounded focal area of fatty change and localized cirrhosis. The fibrotic portal radicles can be distinguished as the dark streaks within the lesion. Photograph of histologic section slightly enlarged.

outline of the stainable droplets. This unstained crystalline material was doubly refractile when viewed with crossed Nicol prisms.

Case 2. B. F., obese white female aged 57 years, entered the hospital with fever of 2 days' duration associated with vomiting and severe epigastric pain which radiated to the scapulae. The patient was known to have diabetes melitis. Physical examination disclosed marked rigidity and tenderness over the gallbladder area. Blood pressure determination was 160 mm. mercury systolic and 90 mm. diastolic. The temperature was 99.4°F. Significant laboratory findings were: blood sugar 224 mgm. per cent, glycosuria 2.25 per cent,

acetonuria and a urine specific gravity of 1.035. Clinical diagnoses were: (1) acute cholecystitis, (2) diabetes melitis and (3) hypertension. Under dietary management, insulin therapy and local measures directed to the gallbladder area, some improvement was noted. Biliary colic recurred, however, and 3 weeks later, under gas and ether anesthesia, the gallbladder was incised and drained and a biliary calculus removed from the ampulla of Vater. The

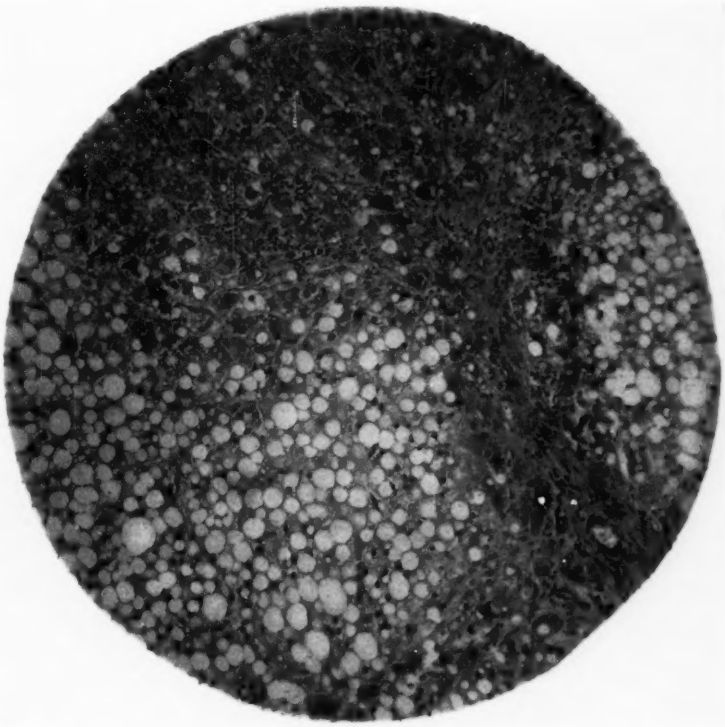

FIG. 3. Case 2. A field within the lesion illustrated in figure 2 showing combined fatty changes and cirrhosis. Photomicrograph circa 50 ×.

convalescence was unsatisfactory owing to loss of fluids and alimentary secretions by incessant vomiting and death occurred shortly after.

Post mortem examination. (Performed 2 hours after death.) The combined gross and microscopic diagnoses were: (1) focal fatty change of liver; (2) recent laparotomy for cholelithiasis and cholecystostomy; (3) chronic suppurative cholecystitis; (4) paralytic ileus; (5) acute nephrosis; (6) hypostatic congestion of lungs with gray infarction at right base.

Description of liver. The liver was soft in consistency and had many adhesions in the gallbladder area. Beneath the mottled left lobe was a sharply circumscribed round, pale, greasy area 2.5 cm. in diameter with a somewhat irregular outline. A few smaller, similar areas were associated with this lesion but none were observed in the other lobes. The remaining liver substance was pale with indistinct markings.

Microscopic description of liver. Throughout the liver, away from the fatty areas, the portal radicles were slightly enlarged, fibrotic and occasionally infiltrated with small round cells, the prolongations of connective tissue partly encircling the lobules. In the region of the central and sublobular veins the hepatic cells were degenerated, necrotic and finely vacuolated but elsewhere in the lobule they appeared well preserved and only rarely contained a single large vacuole. The Kupfer cells were prominent due to their high content of small brown granules and fine needle-shaped pigment crystals.

The localized fatty areas described grossly in the left lobe were sharply circumscribed but not encapsulated (fig. 2). Along the line of demarcation, which traversed different segments of the lobule, there was a border of finely vacuolated hepatic cells compressed into thin parallel cords and separated by congested sinusoids. Throughout the fatty area the hepatic cells contained large sized vacuoles which practically replaced the cytoplasm imparting a signet ring appearance. Most of the nuclei appeared well preserved but some of the cell boundaries were attenuated and broken. The sinusoids were narrow and bloodless. The portal radicles showed proliferating small bile ducts, inflammatory cells, hyperemic blood vessels and an increase of connective tissue with active invasion of the lobule (fig. 3). Bordering the portal triads some hepatic cells were necrotic. Much of the cytoplasm of the hepatic cells in this region contained small acidophilic lumpy hyalin masses identical with the acidophilic cytoplasmic reticulum characteristic of diffuse cirrhosis of the liver, the so-called "alcoholic hyalin." Some nuclei were small and multiple, others large, irregular and hyperchromatic.

LITERATURE

The earliest historical reference we have found to focal fatty changes in the human liver is a case report by H. M. Biggs, presented before the New York Pathological Society in 1891, during his term as president. It is difficult to classify the lesion reported by this observer, and doubtful if it properly belongs with the type under discussion. Nevertheless the reference is included for the sake of completion and historical significance. The liver specimen exhibited by Biggs had numerous light areas which showed distinctly through the capsule, were scattered diffusely through the organ and projected above the cut surface.

Microscopic examination showed these areas to be liver tissue surrounded by dilated vessels and extensive fatty change.

The first apparently genuine example of the lesion encountered in human beings was described by Hugenin. This observer divided focal fatty change of the liver into two main groups with distinct clinical and morphological features. The first group included healthy individuals and animals coming to autopsy after traumatic death. The liver surface showed soft, white, irregular but sharply circumscribed areas, triangular on the cut surface. These areas were mostly situated on the diaphragmatic aspect of the organ on either side of the suspensory ligament. The fat was restricted to the lobular design so that the lobules were either completely free or completely involved. Exudation, pigmentation and nuclear changes were not observed and vascular disturbances were absent both within and about the fatty fields. Nile blue sulphate, Sudan III and osmic acid stains demonstrated neutral fat within the cells. The genesis and fate of the areas remained questionable. Hugenin's second type occurred both in man and animals with infectious diseases and diffuse cloudy swelling of the liver. The characteristics of this lesion were cellular necrosis with interlobular lymphocytic infiltration and an absence of lobular arrangement with respect to the neutral fat. Such areas with hepatic cell damage and round cell infiltration were considered incapable of complete restitution and it was believed they might eventuate in circumscribed cirrhosis.

The next report was furnished by Cesaris Demel who described 3 cases of what he considered a new type of lesion, a so-called "adipose infarct." These "infarcts" were subcapsular, rounded superficially and cuneiform on the cut surfaces. They were pale, grayish yellow, turbid and opaque, with an increased consistency. Nearly every cell in the area contained large droplets of neutral fat. There was no necrosis and the connective tissue elements were well preserved but in several foci lymphocytic infiltration was noted. Thrombotic obstruction of the vessels was not demonstrated. Throughout the liver the outstanding feature was diffuse interstitial hepatitis with prom-

inence of the Kupfer cells. Clinically, the patients were anemic young males who suffered some anatomic cardiac alteration with consequent functional circulatory changes. Cesaris Demel believed the fatty change depended upon arterial compression by cellular infiltration of the prolongations of Glissons capsule together with diminution in sinusoidal caliber by swollen, proliferated Kupfer cells. Therefore local stasis, anemia sinusoid compression and cardiac decompensation all contributed to the production of the fatty areas.

Tedeschi, Marras, Domenichini and Simon reported respectively 4, 2, 3 and 1 cases, the latter's paper being the first to appear in English medical literature. Simon differentiated the condition from lipoma of the liver, with which it may possibly have been confused by early observers, regarding it as doubtful if an indubitable case of liver lipoma exists in the literature. No new etiological theories of importance were advanced in these reports. Three patients were children and 5 young males. The lesions were situated in the left lobe and in the anterior margin and convex surface of the right lobe near the falciform ligament. Otherwise the gross and the main microscopic features resembled those described by Cesaris Demel, there being an abundance of fat droplets in all. Four cases showed infiltration by lymphocytes and polymorphonuclear leukocytes with slight stromal reaction in the involved area. In 2 cases, although the liver cell cords were swollen and difficult to distinguish, the hepatic capillaries correspondingly compressed and, in 1, the arterial branches slightly thickened, yet no evidence of necrosis was observed and the Kupfer cells showed no visible abnormality. Two patients studied by Marras had circulatory stasis and the local mechanism of production of the fatty areas was considered to be ischemia due to nervous spasm of small blood vessels in the presence of deficient collateral circulation. No thrombosis of any of the 3 vascular components of the liver was observed in any instance.

It has long been recognized that slaughtered cattle rather frequently exhibit localized yellow areas in the liver and similar deposits have also been observed occasionally in the dog, hog,

sheep and cat. The first description of these was made by M'Fadyean in 1891 and was later included in Kitt's textbook of Pathological Anatomy of Domestic Animals. The observations of M'Fadyean, Kitt and Claussen, together with the later work of Nieberle-Cohrs and Bugge and Hemmert-Halswick, has recently been reviewed by Bachman. The latter author also studied specimens of liver from 200 healthy cattle, 50 healthy calves and 72 cattle with some disease. Focal fatty deposits were encountered in 61.2 per cent of the normal livers and showed no absolute increase of frequency in the pathologic specimens. Age, sex, and nutrition had no influence upon the incidence. The fatty spots were subcapsular, projected slightly above the surface, had an increased consistency and showed a predilection for the right lobe of the liver. Where they were situated near or beneath the ligaments of the liver, the overlying serosa was smooth but a localized perihepatitis with peritoneal adhesions occurred in all other situations. Regularly these peritoneal adhesions and ligaments contained small net-like blood vessels which extended for a depth of 2–6 cms. below the surface. Although compression was followed by expression of blood into the vessel, the normal direction of blood flow could not be determined nor could their identity as veins or arteries be established histologically. The location and redistribution of the fatty areas were most significant. They showed a predilection for the right edge of the liver, particularly the insertion of the right triangular ligament, the base of Spigel's lobe, the attachment of the duodenal ligament, and the intestinal surface of the left lobe underlying patches of perihepatitis. Microscopically, degenerative changes were only found in areas with severe fatty changes and were considered secondary to this cause. The capillaries were compressed, the interstitial tissue occasionally obscured and the Kupfer cells free of fat. The fatty margins passed directly through a lobule and were not restricted to a lobular design. The injection of the fine vessels in the adhesions confirmed the subcapsular distribution and demonstrated that only very fine branches entered the liver parenchyma.

The experimental production of focal fatty areas has been an incidental finding in 2 different sets of experiments. The first was reported by Ljvraga who, while studying experimental diabetes in the dog, from which the medulla of both adrenal glands had been destroyed by curettage, incidentally noted wedge-shaped fatty areas in the liver. Confirmation of this observation was obtained by employing similar experimental procedures in 6 additional dogs in which identical hepatic lesions were produced 5 months after operation. The fatty areas were single or multiple, sulphur yellow in color, from pin-head size to 1 cm. in diameter and glistened through the unchanged capsule, being slightly elevated and penetrating a millimeter or so into the parenchyma. On a plane perpendicular to the surface the conformation was roughly wedge-shaped with the base toward the capsule. They were distributed near the inferior border and on the upper surface of the right lobe, on the lower surface of the left lobe and in the immediate neighborhood of the gallbladder. Each cell in the area appeared completely filled with lipoidal material, the nuclei being always well preserved although displaced peripherally and the cell body appearing rounded as though under tension. In all cases the glycogen content of the liver was reduced, the hepatic sinusoids congested and, at autopsy, the adrenal glands were contracted and indurated. Ljvraga postulated a disturbance in fat metabolism occasioned by loss of adrenal medullary substance with the production of a state somewhat analogous to disturbed carbohydrate metabolism in the pancreatectomized dog. A primary slowing of circulation was thought to occur, followed by a decrease of oxygen exchange in the liver cell with resulting fatty changes, the formation of fatty areas in the immediate neighborhood of the anterior edge of the liver being favored by impaired circulatory conditions in a congested liver.

Cantarow, Stewart and Housel also observed the occurrence of focal fatty changes experimentally in the livers of 5 dogs subjected to acute hyperparathyroidism induced by intramuscular injection of 100–300 units of parathyroid extract 4 times daily for 3 days. Food was withheld but water was allowed. Two

animals were rendered anemic and in one an attempt was made to reduce the protein concentration of the blood by plasmapheresis and protein restriction. Irrespective of the treatment, the focal fatty areas were essentially similar in all the animals except that they varied in number. Grossly there were pale yellow, firm, sharply circumscribed areas up to 2 cm. in diameter present on the posterior surface around the portal fissure, chiefly occupying the tips of the lobes, but seen also on the surface near the gallbladder and at the site of the peritoneal reflection. The liver markings were regularly maintained in these pale yellow areas. Microscopically the appearance of the liver away from the fatty areas varied in different animals. In some there was slight degeneration of hepatic cells in the center of the lobules and a suggestion of calcium deposition in the bile duct epithelium. There were scattered groups of 2–25 degenerated, acidophilic hepatic cells at times, associated with slight inflammatory cell infiltration, and 1 animal showed periportal and connective tissue proliferation. A relatively large amount of stainable lipoidal material was contained in the hepatic cells, but little or none in the Kupfer cells, both these cell systems containing variable amounts of doubly refractile material in different cases. The focal fatty areas in these animals varied in shape and size, several being roughly square on section. The surface was at times elevated, the lesion appearing somewhat swollen. These fatty areas were very sharply demarcated, the zone of transition between them and the surrounding parenchyma consisting of not more than 1–2 hepatic cells in width. The adjacent hepatic cells were compressed. The fatty change involved uniformly and completely every hepatic cell within the lesion, the vacuoles varying in size and the nuclei, all of which appeared viable, presenting a typical signet appearance. The fatty areas did not have a lobular distribution, comprising in addition to entire lobules, varying sized portions of adjacent lobules, the very sharp line of demarcation bearing no consistent relation to central vein or portal radicles. There was no evidence of calcification or thrombosis. In sections stained with Nile blue sulphate and scarlet red, the focal fatty areas stood out sharply,

every cell in the area being completely filled by a large globule of fat in which occurred many brown, needle-shaped crystals. These areas were extremely sharply demarcated from the surrounding parenchyma. This sharp demarcation was particularly striking when viewed under crossed Nicol prisms, the lipid-containing cells also containing large quantities of doubly refractile material which stood out prominently against the background of the non-refractile surrounding parenchyma.

COMMENT

It is clear from the foregoing that focal fatty deposits in the liver occur under widely different circumstances. Having much in common morphologically, they yet exhibit certain individual characteristics which may ultimately prove to be of fundamental differential significance. In size they are relatively constant, with an average diameter of 15 mm. with variations of from less than 1 mm. to a surface area 30 x 60 mm. They tend to be yellow, firm, superficial, and single in occurrence, the preferred site being the right lobe of the liver near the anterior inferior margin and the falciform ligament. Exceptions to all these features have, however, been recorded. Thus, any lobe of the liver may harbor the lesion, some are white, others soft or unchanged in consistency and multiple areas have been observed. Multiplicity is the rule in cattle and dogs, 1 or more areas lying deeply in the parenchyma. Externally, the appearance is of a circular, or occasionally square, quadrilateral or irregular area exceedingly sharply demarcated and almost invariably wedge-shaped or oval on section. The overlying capsule of the liver is sometimes smooth and unthickened but in cattle there is frequently a patch of perihepatitis and in both our cases there were extensive hepatic adhesions.

Microscopically practically every hepatic cell in the area is distended to capacity with lipoidal material which imparts a typically signet ring appearance in paraffin sections. One observer also reported partial glycogen depletion. The lipoidal material is usually said to consist exclusively of neutral fats but we have demonstrated large amounts of doubly refractile

material by crossed Nicol prisms. A lobular distribution of the lipoids has been described. In the majority of instances, however, a restriction to the lobular design has been absent, the fatty areas comprising, in addition to entire lobules, varying sized portions of adjacent lobules, the line of demarcation bearing no consistent relation to central vein or portal radicle. The transition from signet ring forms to non-fatty cells is abrupt, consisting of not more than the width of 3 or 4 liver cords showing rapidly diminishing vacuolization. The sinusoids enclosed within the area are in most instances compressed by swollen hepatic cells and bloodless. Thrombosis has not been observed in any of the vascular components of the liver. The Kupfer cells are usually unchanged except for slight pigment deposit but in 2 of Cesaris Demel's cases they were numerous and swollen and appeared to obstruct the sinusoidal circulation. In dogs subjected to medullary curettage of the adrenal, hepatic congestion regularly preceded the development of the fatty process. Although varying grades of fibrosis have occurred within the area, no example has been reported which exhibited a surrounding capsule.

The hepatic cells, aside from their high content of lipoidal material, often appear otherwise relatively unchanged and well preserved or, at least, show degenerative changes incident to the causes of somatic death in no greater degree than the surrounding parenchyma. The portal areas are usually inconspicuous, being somewhat obscured by the swollen parenchyma. In a few instances, however, degenerative changes and cirrhosis occurred. Our own cases developed in association with so many diverse complicating factors including hepatic adhesions that an etiologic interpretation is out of the question, at least at present. Although unwilling to attempt an evaluation of the significance of the varied and apparently unrelated findings in our cases, we do wish to emphasize the difference in morphology of the lesions. One presented a simple fatty process without visible abnormality in blood vessels, Kupfer cells or portal areas except for slight compression incident to the swollen state of the hepatic cells. In the other case the picture was complicated by unmistakable

signs of degeneration, hepatitis and cirrhosis. Whether these differences indicate separate and distinct processes unrelated to each other or merely different stages of the same process, together with the question of their etiology are as yet unsettled. The cirrhotic change has all the characteristics of the lesion usually classified as alcoholic cirrhosis, many of the hepatic cells within the lesion exhibiting the so-called "alcoholic hyalin" in abundance. The absence of transition stages between the non-cirrhotic and cirrhotic types, particularly in cattle, is advanced as negative evidence of any progressive relationship between the two.

The uncomplicated focal fatty process in individuals and animals coming to autopsy following traumatic death shows a significant distribution in relation to the ligaments of the liver and has been explained upon a purely mechanical basis (Hugenin, Bachman). Only occasionally is a round cell infiltration noted in the presence of severe fatty degeneration as an apparently secondary feature. The main disparity between the lesions of this type, as observed in man and lower animals, is a restriction sometimes of the lipoidal material to the lobular design of the liver. Etiologic theories have variously attributed the localized fatty deposits to the effects of increased blood supply (Bugge and Hemmert-Halswick), nervous ischemia (Schantz), vascular stasis (Claussen), and an extra-hepatic blood supply derived from vessels traversing the hepatic ligaments and hepatic adhesions. As a clue to their causation Bachman emphasizes their subcapsular situation and localization where hepatic ligaments and perihepatic adhesions may exert a mechanical strain upon circumscribed subcapsular areas of liver tissue. This strain, he reasons, forces the area to yield with resulting disarrangement of the vessels within, particularly the portal vein branches. The exclusion of portal blood from the area creates the need for a new circulation which is accommodated by vessels in the overlying ligaments and adhesions. This alteration in blood supply, with the portal vein branch excluded, together with the low blood pressure in the area under tension, lead to secondary circulatory effects which, themselves, predispose to

fatty changes in small circumscribed peripheral regions of the liver. The conical shape and sharp delimitation of the lesions can also be explained on this basis. The peritoneal vessels are, according to Bachman, rendered more distinct over the fatty area, due either to increased resistance to blood flow offered by the swollen hepatic cells or to an attempt to compensate for increased pressure within the area.

Not much light has as yet been shed on the pathogenesis of the lesion by experimental reproduction of it. The induced process is considered identical morphologically with that occurring spontaneously as described in the medical and veterinary literature. Ljvraga believed the etiology to be circulatory stasis and diminished oxidation by hepatic cells. Too little, however, is known at present of the finer ramifications of the hepatic circulatory apparatus to account for the distribution of the fat according to the lobular design of the liver in some cases and independent of the lobule in others. Since frequently all the cells within the area are viable, the term "infarct" is obviously a misnomer and yet it is difficult to explain the extremely sharp delimitation and absence of lobular distribution on other than a circulatory basis. The situation of the lesions about the hepatic ligaments may offer a clue to immediate causation, but whether their development in experimental acute hyperparathyroidism and adrenal demedullation is significant clinically remains for future observations to determine.

Equally difficult to explain is why cirrhotic changes develop to complicate the fatty condition in certain specimens. One hypothesis states that the degenerative and fatty changes, portal fibrosis and leukocytic infiltration represent a purely local affair not shared by the remaining liver tissue (Hugenin, Cesaris Demel, Tedeschi, Marras). The causes were assigned to different conditions, singly or in combination, such as anemia, cardiac decompensation, toxic or infectious diseases, and vascular stasis due to local nervous ischemia, impaired collateral circulation, arterial constriction by portal infiltrations or diminution of sinusoidal lumens by proliferating Kupfer cells. All these, at least the causes that rest upon a morphological basis,

are so commonly encountered in clinical medicine and experimentally that their significance in this connection is doubtful. An alternative hypothesis which we subscribe to as possibly explaining the pathogenesis of the focal limitation of the fibrosis and alcoholic hyalin is that they represent a residual cirrhosis previously distributed more diffusely throughout the liver. This opinion also receives support from Hugenin's observation that the entire liver is sometimes the seat of parenchymatous degeneration. Possibly, regeneration occurred in the course of time except in occasional areas where the lesions happened to be more severe than in others and it is those areas which still show the residual fatty deposits and old alcoholic hyalin. If this is true, probably originally the whole liver was more or less like those areas.

SUMMARY AND CONCLUSIONS

Two cases of focal fatty change of the liver are reported, the one complicated by cirrhosis, the other uncomplicated, together with a review of the medical and veterinary literature dealing with these localized changes.

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CYSTADENOMA LYMPHOMATOSUM*

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Since 1910, when Albrecht and Arzt published a report of two cases in which an unusual tumor was removed from the region of the parotid gland, quite a number of similar accounts have appeared in the literature from time to time. A total of fifty-four cases are found in the bibliography emanating from foreign and domestic periodicals. In 1933 Kraissl and Stout[1] reviewed the literature and cited nineteen cases reported by various authors, the first being dated 1898. In 1935 Carmichael, Davie and Stewart[2] reviewed the literature and brought the total number to forty-one. It is difficult to estimate exactly how many of these tumors bore the title of branchiogenic cysts because of the conception of many that cystadenoma lymphomatosum is of branchiogenic origin. Examples of this are new growths reported by Hildebrandt[13a] and Sultan,[13] where the former described a neoplasm along with twenty cysts and fistulas of the neck, all of which were considered of branchiogenic origin. Sultan included his own among twenty-two parotid cysts.

Since 1935, when the review by Carmichael, Davie and Stewart appeared, several additional cases have been reported. Wood,[3] in 1935, described three, all of which occurred in males, 37, 48 and 71 years of age. These tumors were approximately of the same size and shape and were well encapsulated. Hall[4] in the same year reported one in a 48 year old male. In this instance the tumor was of one year's duration and was both hard and tender. In 1937 Harris[5] published a report of two tumors of this type; both were in elderly individuals and the relation to the

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parotid gland was practically identical to others recorded in the literature. It is a singular fact that out of all of these growths only two, as mentioned by Carmichael et al., recurred after surgical removal.

REPORT OF CASE

The patient was a white male aged 63, who, a year ago, noticed a swelling in the neck along the anterior edge of the superior portion of the left sterno-cleido-mastoid muscle. The swelling was approximately the size of a lead pencil in thickness and 4 cm. in length, not painful, but because of the gradual increase in size a physician was consulted. Upon physical examination, a mass along

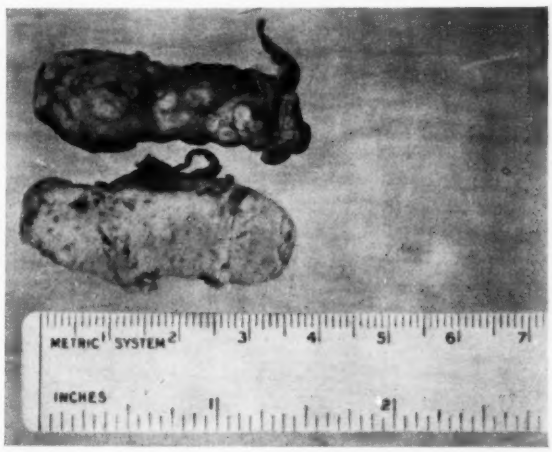

FIG. 1. HEMISECTION OF TUMOR ILLUSTRATING NODULARITY AND CYSTIC APPEARANCE OF SECTION SURFACE

the anterior margin of the left sterno-cleido-mastoid muscle was seen which was not attached to the surrounding tissue. It was soft and fluctuant and drained a brown serous material. The tumor was removed by Doctor W. M. Greig by blunt dissection. No difficulty was experienced in separating the mass from its environment.

Gross specimen. Examination of the growth revealed a mass measuring 4 x 3 x 1 cm., resembling a pecan in shape, firm in consistency. The tissue is light gray to dark gray in color and somewhat nodular in outline. Surrounding the mass is a fibrous capsule. At one pole is a thin-walled cyst 3 mm. in diameter. On cut section, the surface is granular in appearance and varies from light to dark gray in color. Scattered through the tissues are several small cystic areas which exude cheesy material on pressure.

Histologic findings. The capsule consists of fibrillary connective tissue, continuous with fibrous septa found throughout the mass. Between the septa are tubular glands supported by a lymphoid stroma. The diameter of the glands varies from 15 to 500 microns. They are lined by two layers of cells which in places are growing in papillary formation. The basal layer is formed by a tall cylindrical cell whose nucleus lies near the basement membrane. Alternating with the basal layer is a shorter cylindrical cell that borders on the

FIG. 2. PHOTOMICROGRAPH OF TUMOR TO SHOW GENERAL TOPOGRAPHY. THE CYSTS ARE CLEARLY SEEN. THE DARK AREAS REPRESENT THE LYMPHOID STROMA

lumen. The nuclei are large, round and granular and each has a distinct nucleolus. Between the cylindrical epithelium are secretory capillaries that extend along the length of the cells to widen as the lumen is approached. Stained with Mallory's aniline blue-orange G-fuchsin, the capillaries are shown to be occupied by a homogenous deep purple red substance. The tubular glands contain an amorphous material. The stroma is lymphadenoid in type with an occasional germinal center.

The purpose of this paper is not to attempt a recapitulation of the material reviewed by Carmichael, Davie and Stewart, or Kraissl and Stout, but to bring up to date the literature of this rare tumor and endeavor to clarify some of the conceptions regarding its origin.

This tumor first attracted attention in 1898 when Hildebrandt discovered a case which he considered to be closely related to

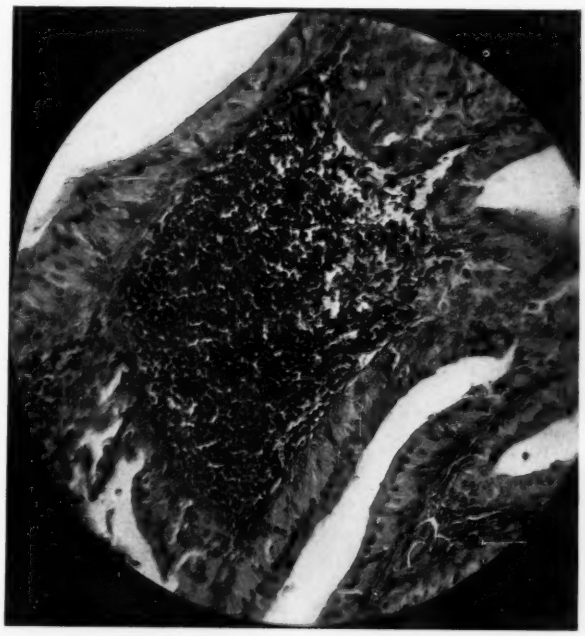

FIG. 3. LOW POWER PHOTOMICROGRAPH OF TUMOR TO SHOW THE LYMPHOID STROMA BENEATH THE GLANDULAR EPITHELIUM

branchiogenic cysts and therefore of branchiogenic origin. In the same year Sultan found one of these tumors and interpreted it merely as one form of parotid cyst. Lecene and Morestin also thought that the tumors that they each saw originated from out-pouching of pharyngeal ectoderm or branchial pouches. Next in line, according to the date of discovery, came Albrecht and Arzt,[6] who ascribed the origin of the tumor to misplaced

epithelial tissue from the entoderm and mesenchymal germ layers or to the inclusion of epithelium of the entoderm of the oral cavity into lymph nodes. Glass took a similar view.

Ribbert,[7] however, interpreted Glass' explanation in the following way: "The lymphoid tissue and parotid lobule grew into one another and the mixed portion became separated from the parotid gland." In so far as this process takes place in

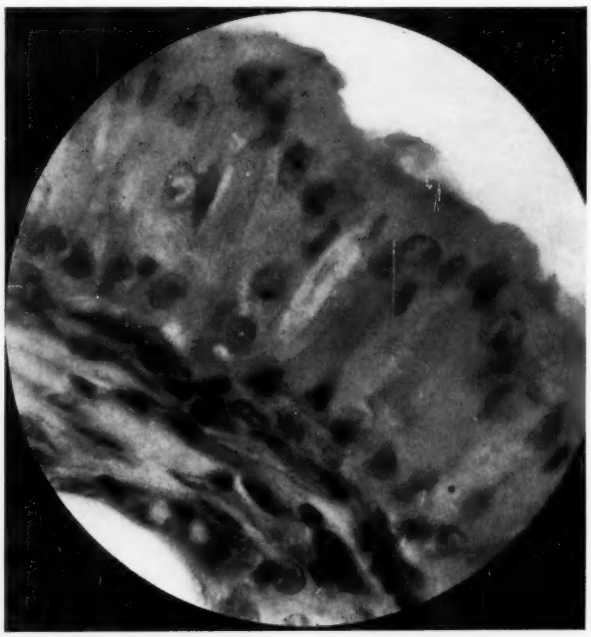

FIG. 4. HIGH POWER PHOTOMICROGRAPH OF TUMOR TO SHOW THE SECRETORY CAPILLARIES BETWEEN THE CYLINDRICAL CELLS LINING THE GLANDS

the development of the branchial cleft, Glass prefers to call the tumor a branchiogenic cystadeno-lymphoma.

Neumeister at the same time said practically the identical thing but left the question open as to whether the entodermal and mesenchymal tissue were misplaced at the same time and a lymph node developed from the mesenchymal tissue at a later date, or whether the entodermal epithelium arrived in a site from

which lymphoid tissue would later develop. Askanazy believed the most probable origin to be from the branchial clefts. He thought that the cells closely resembled the eosinophilic cells of the parathyroid gland. Then according to Carmichael several investigators, including Nicholson, Menetrier, Peyron, Surmount and Stober, thought that the tumor might have arisen from preparotid lymph nodes, in which Neisse had been able to demonstrate scattered acini of salivary tissue and in some cases lobules of salivary tissue which contained excretory ducts. In explaining the presence of these glands, Neisse showed that early in the development of the human fetus (120 mm.) there are lymph nodes in the parotid and submaxillary glands which have in their substance salivary tissue. At a later date these glands become the preparotid lymph nodes, at which time they are encapsulated and free from the parotid. It is entirely possible that when these lymph nodes moved away from the parotid gland they might still have retained portions of salivary tissue in their substance for varying lengths of time. At times these salivary structures in preparotid lymph nodes have been found in adult life. The next step in the supposition would be the development of tumors from these gland structures.

In 1929 Warthin[8] reported two cases. Because of a similarity that he saw between the epithelium of the tumor and the cells lining the Eustachian tubes, he thought that the former could have originated from a dystopia of the pharyngeal ectoderm. In the following year Wendel[9] substantiated Warthin's views.

In 1931 Hamperl advanced the observation that as an individual grows older there is a type of cell found in salivary glands which resembles the epithelial structure of the tumor more closely than it does the normal epithelium of salivary glands. These cells were described by Schaffer (1897) and then studied by Zimmermann[10] and termed pyknocytes. In 1932 Jaffé[11] reported a case. He felt, after careful study, that the epithelial cells of the tumor corresponded in every way with the onkocytes of Hamperl. Because of this Jaffé proposed the name onkocytoma. His explanation of the lymphoid tissue was that it was the remnant of a lymph node and the presence of the germinal

centers was in answer to the greater resorptive demands of the tumor because of its secretory products. This term, onkocytoma, has met with opposition on the part of Harris who thinks, and we believe rightly, that because of the fact that onkocytes are an evidence of senility and are found only in individuals at least past twenty years of age, the term does not explain the presence of this tumor in one case of Albrecht and Arzt, which was only twelve years of age, and in the case of Cunningham[12] which was sixteen years old. The other objection of Harris, which could probably be overlooked, is that the term onkocytoma would only add another word to an already overburdened medical vocabulary.

Peyron removed two cystic structures from the parotid region. On microscopic examination, the walls were found to contain Hassall's corpuscles. From this observation and Askanazy's description, one would have to bear in mind the possibility that these tumors might arise from any of the branchial clefts. In this process the supporting structure of the cystic tumor would thus be characteristic of the structures which normally arise from the branchial clefts such as the parathyroid gland and the thymus.

Sternburg mentions two completely opposite views as to the possible origin of gland-like structures in lymph nodes: one view is that they arise by metaplasia from the endothelial lining of the lymph vessels; the other that they arise from embryonal rests. Still another hypothesis is that the glandular epithelium is swept into the lymph nodes from the surrounding structures. One thing in common to all of these theories, however, is that the glands, once they are in the lymph nodes, may give rise to papillary cystadenoma lymphomatosum.

In the publication of Kraissl and Stout the authors tried to explain the origin of the tumor by what they called an orbital inclusion. In order to justify their hypothesis, these investigators reviewed the development of the orbital glands in some members of the carnivora. During this investigation they found that there was a formation of a sulcus from the oral cavity, which finally develops into the adult parotid gland. As has

been reported from time to time, tubular structures have been found in the region of the parotid gland. These were thought to be a parotid anlage, as shown by Weishaupt, and were therefore thought capable of developing into the cystic structures which were occasionally found in the region of the parotid gland. Following this train of thought, Kraissl and Stout then discovered that early investigators in the study of the development of the salivary gland had found a tubular structure lined by epithelium and surrounded by mesenchyme in the region of the parotid gland but not connected with it. They gave this structure the name of orbital inclusion. They pointed out that as the embryo increases in size the parotid gland grows larger and larger and finally lies in close contact with the orbital inclusion. Just exactly what happens to these orbital inclusions in humans has never been determined, but it is only fair to assume that a closed vestigial duct lined by epithelial cells derived from oral ectoderm might at times develop into cystic structures. This supposition becomes more plausible when it is learned that Weishaupt had seen dilatation of one of these structures under the microscope. By this explanation both the lymphoid and epithelial elements of the tumor in question are accounted for. In evaluating this explanation, however, we are at a loss to explain the position of the tumor in our case, which was at the anterior border of the superior portion of the sterno-cleido-mastoid muscle, while the orbital inclusion is situated in an anterior position in relation to the parotid gland.

SUMMARY

A case of cystadenoma lymphomatosum is presented. That it arises from the branchial pouches seems the most probable explanation after all the evidence is taken into consideration. From the fact that some investigators saw what, to them, resembled thymic tissue, and others saw tissues closely resembling acidophylic cells of the parothyroid gland, it would be safe to assume that, at least in two cases, there is some reason to adduce the origin of cystadenoma lymphomatosum from branchial pouches; the particular pouch from which the tumor develops not being the same in every instance.

We are indebted to Dr. W. M. Greig for his permission in reporting this case and to Prof. W. C. Johnson, of the University of Colorado Medical School, for his kindly counsel.

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SYPHILIS AS A FACTOR IN THE BIOSCOPICAL DIAGNOSIS OF ADULT CERVICAL LYMPHADENOPATHY*

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The observation of four cases of enlarged glands in the neck in which syphilitic infection affected the diagnosis seems proper to put on record.

While it is admitted that syphilitic adenopathy may be one of the symptoms of acquired and especially congenital syphilis, its relationship to other affections of the lymph node system has not been emphasized. Jadassohn puts it that generalized lymph gland swellings of syphilitic etiology can simulate Hodgkin's Disease, pseudo-leukemia, splenic anemia, et al. because of their slow growth, painlessness, disassociation with the skin proper and atypical blood findings. Most writers agree that the diagnosis is not at all easy even with all the criteria of clinical diagnosis, and most of them state that treatment alone will answer the question. It is desirable to discuss this further after citation of the cases.

The first case, the records of which have been lost, is reproduced as a photomicrograph in Stengel and Fox Text Book of Pathology, 8th. Edition, 163. The subject of this material was a young person with a chain of painless, adhering glands in the neck; the Wassermann was weakly positive. No other significant data are recalled. The diagnosis was made at once by simple methods of histology and confirmed by the discovery of spirochaetes lying along vessels in the cellular border of the gummata.

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The second case was a woman of 55 with massed glands on the left side of the neck and small nodes on the right, with a history of slow increase in size over three months, very moderate anemia, no demonstrable involvement of the mediastinum or spleen, a differential count in the blood within normal limits and no suggestive history (miscarriages, et al.) of syphilis. The Wassermann was weakly positive. Bioscopic adenectomy revealed solid, pale, grey-yellow glands of the Hodgkin's type, moderate in size, with a heavy capsular covering binding units together. Microscopically, there were areas of acceptable lymphogranulomatosis of the Hodgkin's type and islands of granulation tissue in which periarteritis was prominent. The cellular construction was chiefly imperfect epithelioid cells, numerous plasma cells, irregular arterioles, and some young swollen cells of the fibroblast type. This gland was looked upon as a combination of Hodgkin's Disease and syphilis. The patient did very well under radiation and lived for three years when she was lost sight of.

The third case was a man of 21 presenting a chain of glands on each side of the neck, firm, painless, movable and varying in size from just perceptible to 3 cms. The inguinal and axillary glands were slightly enlarged. The temperature ranged between normal and 99.2. The leucocyte count was 14,000 of which 70 per cent were young lymphocytes. There was no history of venereal disease and no observation of scar nor cutaneous eruption. Duration of the illness was vague but perhaps two months had elapsed since the first perception of enlarged nodes. A tentative diagnosis of acute or subacute lymphatic leukemia was made. Biopsy was done and after this a Wassermann which was strongly positive. Histologically, the node presented a diffuse lymphatic hyperplasia, an increase of perceptible blood vessels with periangiitis and prominent reticulum cells; a few tiny necroses were seen. A diagnosis of hyperplasia and lymphadenitis was made. No attempt to find spirochaetes was made. Antisyphilitic treatment gave the diagnosis. It had only been confused by the type of lymphadenopathy and the blood count.

The fourth case was a colored male of 24 with tuberculous pleuritis, a mass in the mediastinum believed to be a tumor, and an enlarged gland in the right supraclavicular region appearing during the latter part of the illness and believed to be a metastasis. The Wassermann was weakly positive. No autopsy was permitted. Histologically a diagnosis of syphilis and miliary tuberculosis was made on the biopsy of the gland. The latter was represented by recent epithelioid tubercles while syphilis existed as granulation tissue, destruction of small blood vessels and foci that could be called gummata. Some larger necroses existed that were probably caused by a combination of the two pathological processes. Tubercle bacilli were found in the small granulomata, spirochaetes were not found in the granulomata but were discovered by Levaditi and Warthin methods in two places along blood vessels of the granulation tissue.

It is to be admitted that the large necroses might be caseous tubercles or gummata and the observer admits the practical impossibility of stating unqualifiedly that they are one or the other, but believes them to be syphilitic principally because fibroblasts about the border of the necrosis extended well into the mass, because of the extensiveness of the reticulum throughout the necrosis and the penetration of fibrous tissue into it, the retention of the outlines of cells within the degenerated area 'as through a veil', and the acceptable syphilitic granulation tissue in the non-necrotic sections of the gland.

The coexistence of syphilis and some other pathological process in lymph nodes is admitted. The effect of one upon the other has been the subject of some debate without profitable result. Most of the discussion antedated the application of the Wassermann principle to clinical medicine but since it has become a routine procedure the confusion has been less in evidence and discussion less frequent. However, the present report is intended to call attention to the fact that syphilis may be a factor in clinical bioscopical diagnosis so that the clinician and the pathologist should be informed in all cases of the serological state of the patient's blood.

An interesting feature of the examples here presented is that

the adenopathy was most pronounced in the neck. Stokes,[1] Jesionek[2] and Zurhelle[3] believe that anterior cervical lymphadenitis is less to be expected from syphilitic infection than from other diseases whereas syphilitic lymphadenopathy is more apt to effect the posterior cervicals, epitrochlears and inguinals. Anterior cervical adenopathy suggests pharyngeal syphilis. Gumma proper of the lymph nodes in general is variously reported; it appears to occur more often in long standing syphilis, of many years indeed. Fournier saw one in 3429 cases; Lustgarten thinks it is not so uncommon but both of these authorities wrote about their experience before the vigorous treatment of the disease by the arsenicals. However, both Jesionek and Jadassohn quote authorities indicating that there is some resistance in the lymphatic tissue to the virus of syphilis. It is admitted that they react at once when the spirochaete is drained into them from a focus of primary infection and become somewhat enlarged as the infection becomes generalized. They recede however, unless the disease be malignant and, certainly under treatment, may not be discoverable.

There is nothing in the acquisition of syphilis that of itself appears to favor the development of leukemia, lymphomatosis, Hodgkin's Disease or tuberculosis. To be sure, in chronic or untreated syphilis there may be a continued hyperplasia of lymphatic elements that would seem to provide a stimulus for unregulated and unrestrained lymphatic overgrowth.

The opposition offered by the two diseases, one to the other, is also not clear. Judging by the descriptions of Zurhelle and the accounts given above, Hodgkin's Disease and tuberculosis may unfold their recognizable histology side by side with that of syphilis.

The stimulating effect of one upon the other when coexistent is difficult to measure. The destruction of blood vessels by syphilis if rapid, should assist in the development of necrosis, while if slow, it might assist in the progressive character of the granulation tissue to which the Hodgkin's picture may be likened. Etienne claims, and Zurhelle does not disagree, that syphilis may whip up the activity of tuberculosis by the type of granula-

tion tissue, especially along blood vessels, whereby organisms are spread and tissue nutrition is hampered.

The effect of treatment in the presence of coexistent syphilis and some other disease may well be considered. In this respect a paraphrase quotation can be made from Paget and Moore, (American Review of Tuberculosis 1936, 33: 10), that tuberculosis is more important and dangerous than syphilis and that while none of the therapeutic measures currently used for tuberculosis will adversely affect syphilis, some of those used for syphilis may seriously modify the course of tuberculosis. However, it may be that this is not absolutely true and that it would be better to judge each case for itself.

The histological diagnosis of tuberculosis, lymphosarcoma, Hodgkin's Disease, the lymphocytic hyperplasias, endothelioma and reticulum cell sarcoma is not very difficult. The histological diagnosis of syphilis may be very difficult, especially today when syphilitic infection rarely goes undiagnosed and untreated and indeed need not so proceed.

Virchow stated that there is very little that the studious pathologist can find to distinguish between the granulation tissue of the granulomata. It can be added here that the pathologist must take all the factors into consideration and must be helped by clinical records, serological tests, and if possible, the effects of treatment.

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TULAREMIA OF THE HUMAN BREAST*

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As a rather thorough review of the literature has failed to disclose any instance of tularemia of the human breast, the following case is reported, as, apparently, the first on record.

Case Report. The patient, Mrs. M. V. H., age 53, housewife, entered St. Vincent's Infirmary under the services of Dr. S. C. Fulmer. She had been in the hospital in 1935 with menopausal menorrhagia, at which time a dilatation and curettage with application of radium was done.

On this admission January 11, 1937, the chief complaint was fever and general pains, duration five weeks. Just before the onset she had noticed a furuncle on the second finger of the left hand. The next day she became very sick with high fever, generalized aches and pains and drenching perspiration. Two days later the finger became intensely swollen and bluish in color followed by swelling and tenderness of the glands of the left axilla. The fever persisted for four weeks. During the two weeks previous to her entry into the hospital she had been improving, the fever had subsided, and the lesion on the finger and the axillary swelling had shown a definite improvement.

The patient gave the history of having cleaned and cooked rabbits and served them to her family two weeks before the development of the lesion.

Physical examination revealed a well developed very obese female apparently not very ill, blood pressure 104/82. Liver and spleen not very palpable. There was a healing lesion of the left middle finger on the palmar surface of the distal phalangeal joint. The left axillary glands were enlarged and slightly tender.

The right breast was larger than the left, the nipple deviating outward and upward. There was a firm, hard mass 6 centimeters in diameter in the lower and outer quadrant. This tumor was not fixed to the deeper structures, but slightly fixed to the skin, and appeared to be incapsulated. The right axillary glands were not palpable. According to the patient, the breast tumor was first noticed two weeks previous to her entry into the hospital.

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* Received for publication December 16th, 1937.

The leucocyte and differential count showed: 5,400, polymorphonuclears 55 per cent, lymphocytes 34 per cent, monocytes 9 per cent. The blood serum agglutinated B. Tularense completely 1 to 140.

Diagnostic possibilities considered were: (1) Tularemia, (2) Hypertensive heart disease, (3) Carcinoma of the breast.

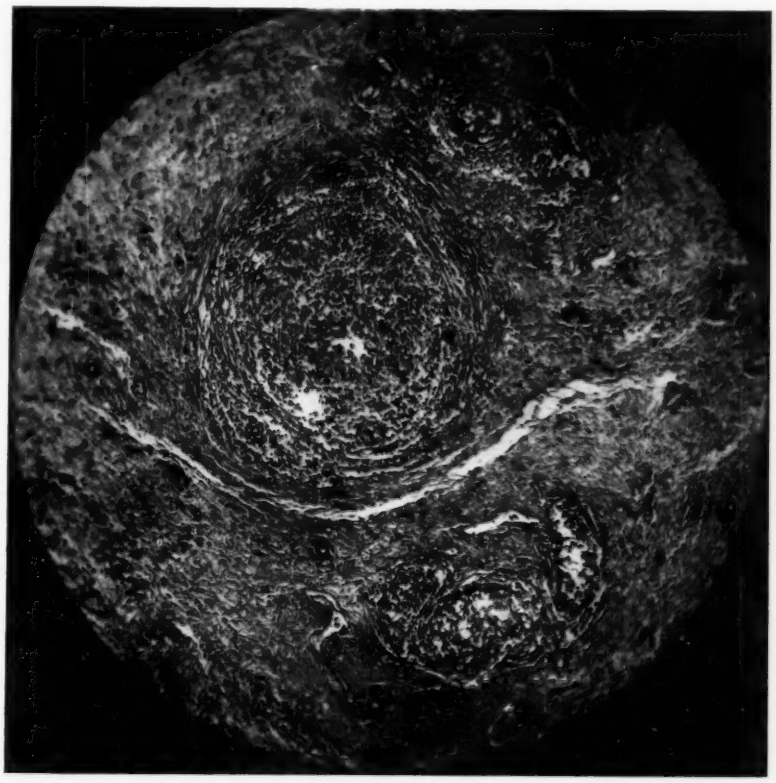

FIG. 1. EARLY LESIONS, TULAREMIA OF BREAST

The surgeon, Dr. George V. Lewis, examined the patient and advised excision of the tumor, microscopic study and, if malignant, a radical breast operation. The tumor was excised and a study of the frozen sections stained by polychrome method revealed an inflammatory lesion. A simple amputation of the breast was done.

Gross examination of the skin revealed the nipple, mammary gland and underlying bed of fat and muscle tissue while the cut surface revealed a marked fibrosis of the mammary gland. There was a tumor mass about 3 centimeters in diameter in the upper right quadrant 3 centimeters to the right of the nipple which cut with little resistance to the knife and left a granular surface and in

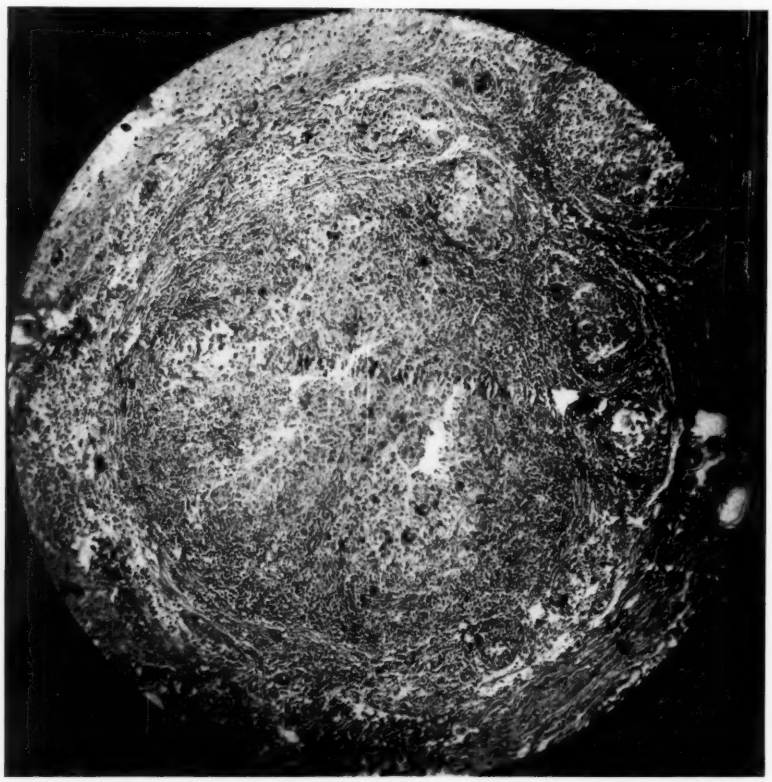

FIG. 2. LARGE, TYPICAL LESION, TULAREMIA OF BREAST

which could be seen small areas of necrosis. The gross manifestations resembled those of cancer.

Microscopic examination revealed many partially circumscribed areas varying considerably in size and appearance. The earlier lesions showed accumulation of endothelial cells, lymphocytes and a few leucocytes surrounded by a thin fibrous wall. The older lesion showed necrosis in the center.

While the lesions resembled tubercles there was a complete absence of giant cells. A diagnosis of tularemia was made from the study of the permanent sections.

At the time of operation a portion of the breast tissue was macerated and injected into the inguinal region of two guinea pigs. Five days later one pig

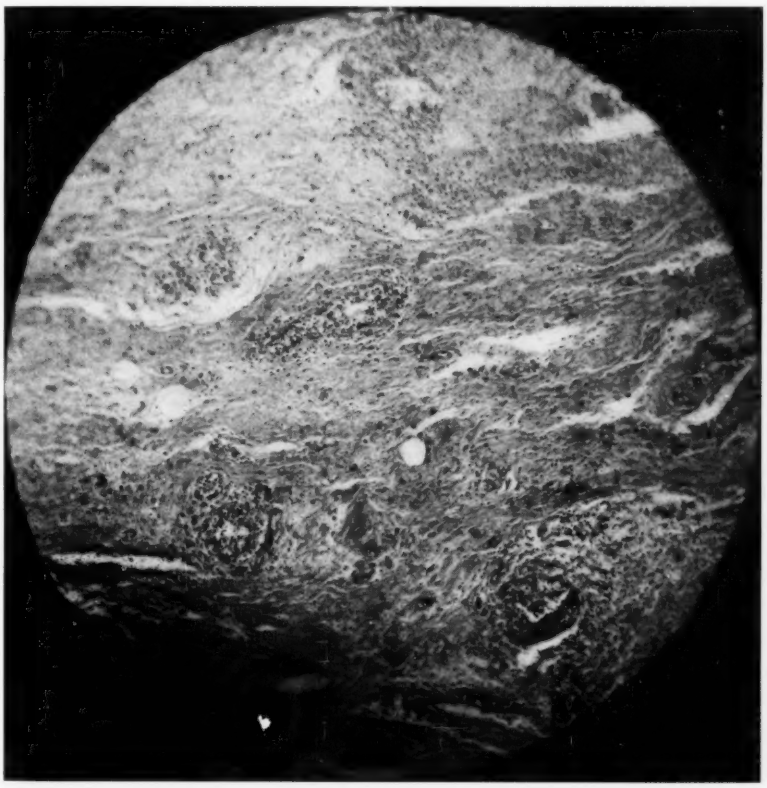

FIG. 3. SMALL LESIONS, TULAREMIA OF BREAST

died and both had very large lymph glands. At autopsy both pigs showed typical lesions of tularemia in the livers and spleens. An attempt to culture the organism was unsuccessful. Two more animals were inoculated; these died in five days. The organism was not obtained. A third group of animals were inoculated and the organism was obtained in pure culture on rabbit's blood-

cystine agar. (I have isolated the organism several times but in each case I have had to carry it through at least three animals before obtaining a culture.) A suspension of the culture was injected into two pigs which died promptly in about four days with lesions of tularemia.

The patient had an uneventful recovery and is living and well at the present time.

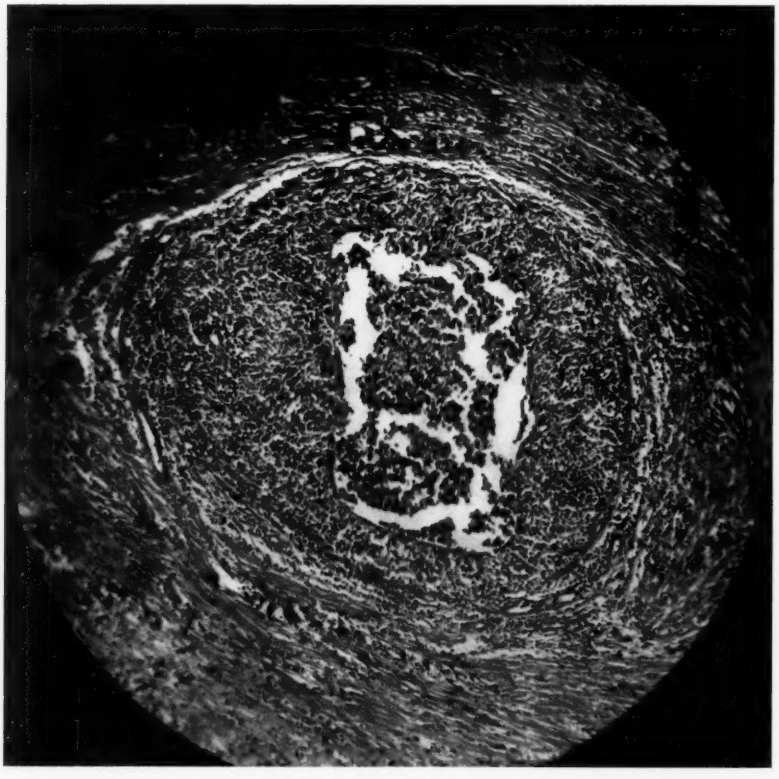

FIG. 4. ADVANCED LESION, TULAREMIA OF BREAST

This case was interesting for the following reasons: (1) The initial lesion occurred on the left hand and involved the right breast so that the organism must have been carried by the blood stream.

(2) Tularemic lesions in human tissue have only infrequently been studied. The lesions are quite typical and should enable the diagnosis of the disease.

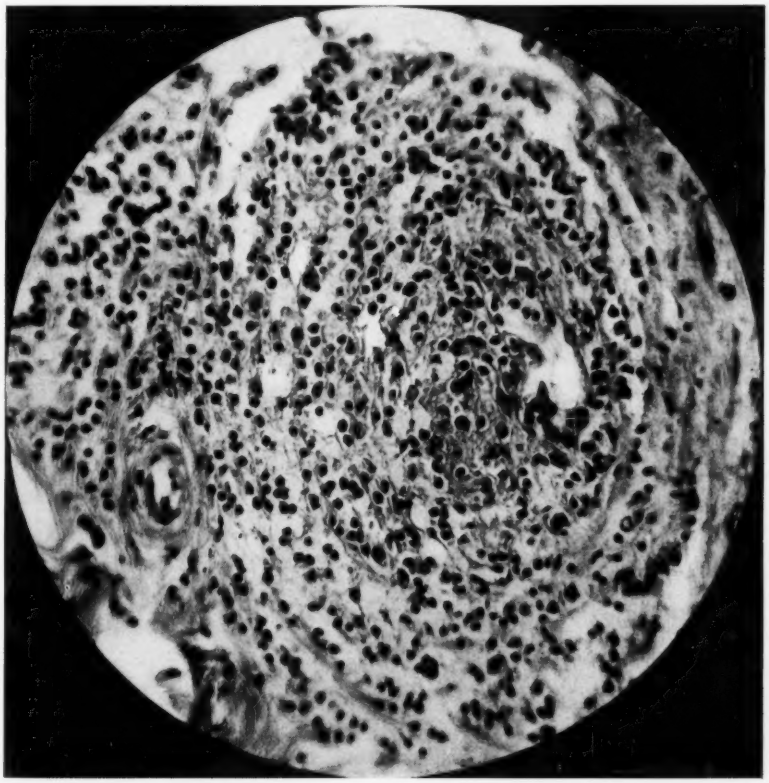

FIG. 5. HIGH POWER, TULAREMIA OF BREAST

(3) A rather thorough review of the literature has failed to reveal a report of tularemia of the human breast.

It would appear that this is the first case of tularemia of the human breast.

DISSEMINATED ECHINOCOCCOSIS*

O. O. WILLIAMS

From the Department of Pathology, University of California Medical School

The incidence of Echinococcus disease in North America is relatively low. Riley[1] states that up until 1933 approximately 430 cases had been reported in Canada and the United States. This low incidence, however, may be more apparent than real as Heller[2] says that most of the busier surgeons of Kansas City and vicinity have encountered one or more cases in recent years. Two relatively large series of cases have been reported by Magath[3] from the Mayo Clinic and by Davis and Balboni[4] from the Massachusetts General Hospital. In their series, as is the case of most of the patients in this country, practically all were of foreign birth.

As is well known, uncomplicated echinococcus cysts may be entirely compatible with comparatively good health. The condition is frequently found in exploratory operations for a mass in the abdomen and may often be encountered as a surprise at postmortem where it was not a causative factor in the patient's death. However, complications of cysts often call for aggressive treatment, as in secondary infections of the cysts, or at least may be a factor in differential diagnosis.

One of the frequent complications is the formation of secondary cysts which result either from spreading at operation or by spontaneous rupture, the latter often occurring in cysts located in an abdominal organ. Dew[5] states that widespread dissemination may result from a spontaneous rupture into a blood vessel.

The following case is one in which widespread early fibrotic and degenerating lesions were found.

* Received for publication September 13th, 1937.

Report of Case

A. G. M., a white woman 53 years of age, entered the University of California Hospital Aug. 2, 1934, complaining of a mass in the neck, severe dyspnea, and recent loss of weight. During her fourth pregnancy the patient developed a small nodule in the region of the thyroid which gradually increased in size. Similar nodules developed twelve years following the first one and were of gradual growth until two and one-half years ago when the increase in size became more rapid. No subjective symptoms were noticed until several months previous to admission, when she began to lose weight, became nervous and irritable, and was easily agitated and fatigued. Five months previous to admission she noticed slight swelling of ankles. Nocturnal asthma manifested itself three months later, and skin lesions developed on neck and thorax one month before admission.

The patient was born in Malaga, Spain, moved to Honolulu when 25 years of age, and to California when 38 years old. Her husband was dead. Her occupation was housework. Her average weight was 170 lbs. and on admission was 122 lbs. She was married at 16 years of age. There were nine pregnancies with three children dying at birth. Menses were regular until two years ago when they began to occur at two month intervals. Her periods occurred every other month until the time of admission.

The patient was a poorly nourished Spanish woman showing no marked discomfort. There was present over face, extensor surfaces of both forearms, and on the anterior thorax, a pale opaque papular eruption which was discrete and had flattened tops and erythematous borders. There was a right lenticular cataract. The left fundus showed dilated veins and thin tortuous arteries. Two large masses were visible and palpable in the neck. The larger mass was soft, cystic, and did not cause choking on gentle pressure. The blood pressure was systolic 175 mm., diastolic 75 mm. The vessels were moderately thickened. The liver extended 4 cms. below the right costal margin, and was smooth and not tender. A large mass was palpable in the left upper quadrant which reached to 3 cms. of the anterior iliac spine and 4 cms. of the midline of the abdomen. It was freely movable, descended with inspiration, was irregular in outline, and had a notched superior border, but could not be definitely identified with the spleen.

The basal metabolic rate was plus 62 per cent on one occasion and plus 50 per cent on a repeat test. The glucose tolerance test was, fasting, 0.104 per cent; first hour, 0.189 per cent; second hour, 0.234 per cent; and third hour, 0.152 per cent. The urine was negative for albumen, showed some copper reduction on one occasion but was negative on a repeat examination, and contained a few granular casts. The red blood cell count was 4,430,000, with a hemoglobin of 78 per cent. The total white cell count was 6,800 with a differtial of 66 per cent P.M.N., 10 per cent non-filaments, 21 per cent lymphocytes and 13 per cent large mononuclears. No eosinophils or basophils were found.

The complement fixation test was negative. An X-ray of the chest showed calcifications in the region of the left lobe of the thyroid gland. There was a peribronchial increase in density which suggested a peribronchitis or diffuse metastatic invasion of the lung. The gastro-intestinal series was negative. Complement fixation or intradermal test for echinococcosis was not done.

A subtotal thyroidectomy was done on August 13, 1934, at which time considerable adherence of the thyroid gland to the surrounding tissue was found. Following the operation the patient became disoriented, the temperature rose rapidly, and the following day she became restless, dyspneic, and cyanotic until her death 36 hours after operation.

The thyroid tissue removed at operation was lobulated and had a thick capsule. The diagnosis was adenomata of the thyroid, adult pattern, with degeneration, fibrosis, and calcification.

Pathological report

The body was that of a well developed and fairly well nourished white woman 53 years of age. The body length was 60 inches and the estimated weight 130 lbs. Over the forearms, upper anterior thorax, anterior abdomen, and the flanks were numerous discrete slightly elevated papules measuring 3 to 4 mms. in diameter. These were attached to the skin. There was a fresh surgical wound measuring 14 cm. in length in the lower anterior surface of the neck. A few palpable lymph nodes were found in the axilla and the inguinal regions. Rigor mortis was moderately advanced and livor mortis present. Edema was absent. The pertinent findings are as follows:

There was no fluid in the abdominal cavity. The liver extended 3 cms. below the costal margin on the right and 5 cms. below the xiphoid process. The spleen was 6 cms. below the left costal margin and a large mass protruded from its lower pole.

The right lung weighed 700 gms. The lower lobe was somewhat congested. The pleura was thickened. The upper lobe of the lung contained numerous small grayish areas 1 to 2 mm. in diameter which stood out from the brownish lung tissue. These areas were not sharply circumscribed but appeared to fade into the adjacent tissue. They were firm to palpation. However, the upper lobe contained air. The left lung weighed 600 gms. The upper lobe was similar to that of the right lung. No evidence of embolism was found. The peribronchial lymph nodes were enlarged. The cut surface showed numerous grayish nodules which were made prominent by the dark pigment surrounding them.

There was an enlargement of the mesenteric lymph nodes, very pronounced around the pancreas and splenic region. The retroperitoneal lymph nodes were also enlarged.

The liver weighed 1850 gms. The surface was finely granular in character. On the antero-lateral surface were two elevated calcified areas measuring 5 x

1.5 cms. and 3 x 2.5 cms. respectively. They were elevated 0.2 cms. above the surface of the liver. The cut surface of the liver showed periportal grayish areas surrounded by light brown tissue. Section of the calcified areas showed a thick outer shell measuring 0.3 cm. in thickness and a fibrous inner wall. The

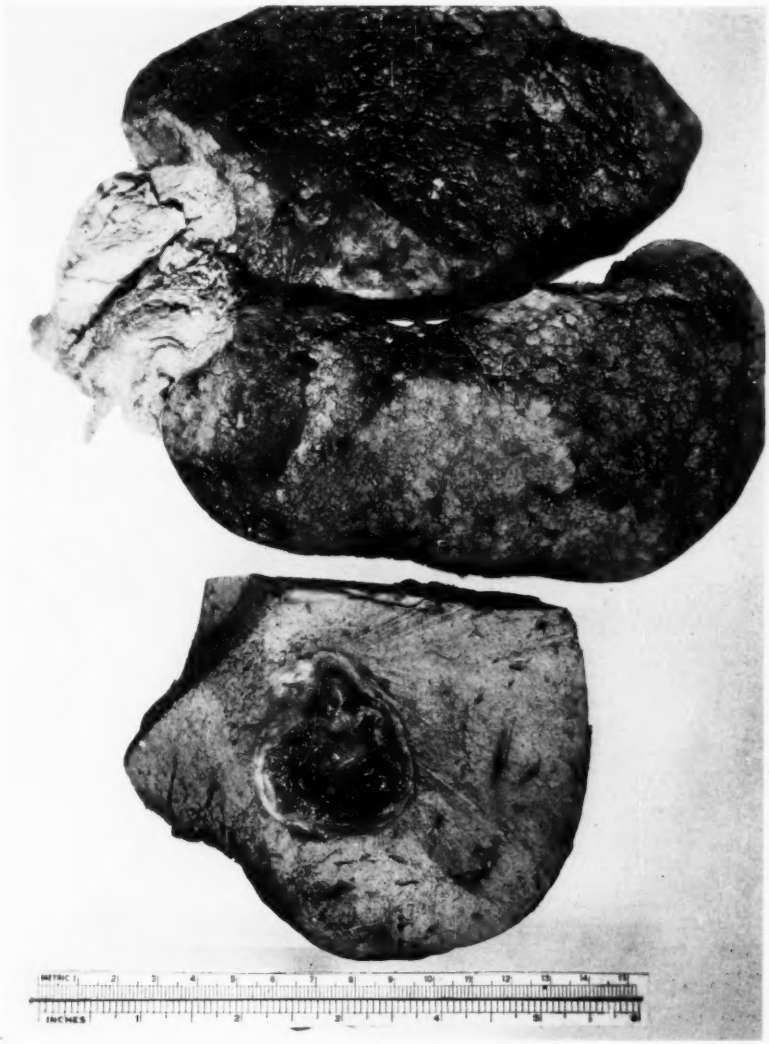

FIG. 1. Showing degenerating cyst in spleen, upper photograph. Lower photograph is one of the healed cysts in the liver.

cysts contained a gelatinous material. The gall bladder contained 30 cc. of dark colored bile easily expressed into the duodenum. The pancreas appeared grossly normal.

The spleen weighed 700 gms. The surface was roughened and granular in appearance. A firm white mass protruded from the spleen at the lower pole. The entire splenic tissue was composed of slightly elevated grayish areas which were surrounded by brown colored tissue. The mass at the lower pole measured 7 x 4.5 cm. and extended into the splenic tissue along the hilus and lower pole.

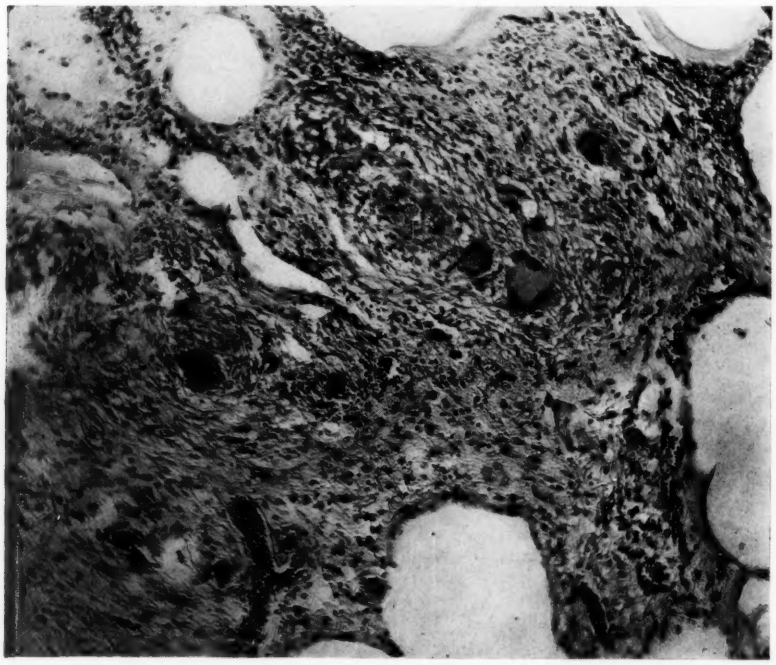

FIG. 2. Section of lung showing giant cells, a fibrotic lesion, and, in the left center zone, a nuclear mass surrounded by dense fibrous tissue.

The inner surface showed degeneration of the capsule with caseation of the contents while the lower half of the mass showed numerous small sac-like bodies.

The genito-urinary system contained no gross lesions. The bone marrow of the middle of the femur was abundant and pink. There were numerous small grayish firm nodules scattered through the tissue. The paravertebral lymph nodes measured from 0.5 to 2 cm. in diameter. There was enlargement of the superficial nodes, the mediastinal nodes, the iliac, and the pelvic nodes. The cut surface appeared to be of uniform texture and was grayish-white in color.

Histologic report

Lung. The pleura was thickened and fibrous in character. There were numerous small tubercle formations beneath the pleura and in the interstitial tissue of the lungs. These varied considerably in their structure, some showing a central necrotic core surrounded by concentrically arranged fibroblasts and an outer zone of small and large mononuclear cells. Large multinucleated cells

FIG. 3. Spleen showing tubercle formation with giant cells, some of which inclose clear spaces or debris. Fibrotic lesions are present.

of the Langhans' type were found on the outer edges of the nodule. Others showed a fibroblastic organization without lymphocytic infiltration or giant cell formation. In other areas giant cells appeared to be surrounding clear spaces. In these areas little fibroblastic reaction was present. The remainder of the tissue showed small atelectatic and emphysematous areas with marked congestion and intra-alveolar coagulated serum.

Liver. The blood vessels of the thickened capsule showed marked conges-

tion and there was lymphocytic infiltration beneath the capsule. The interlobular fibrous tissue was increased in amount and small fibrous strands extended into the lobule for a short distance. The liver cells showed cloudy swelling and vacuolation. A slight central necrosis was present. In the interlobular fibrous tissue were numerous small nodules composed of central areas of fibrous tissue and peripheral zones of lymphocytes with occasional peripherally located multinucleated giant cells. The cyst wall was densely fibrous and was surrounded by fibroblastic reaction, lymphocytes, and occasional giant cells. Groups of liver cells showing degenerative changes were surrounded by edematous fibrous tissue.

Spleen. The capsule and trabeculae were thickened. The splenic structure was obscured by numerous tubercle formations with a structure similar to those found in the lungs. Only an occasional Malpighian body remained. The fibroblastic reaction was marked throughout the spleen.

There were no significant findings in the pancreas, gastro-intestinal tract, kidneys, adrenals, uterus, ovaries and aorta.

Lymph nodes. The peripheral, abdominal, and peribronchial lymph nodes showed massive involvement with numerous small tubercle or nodular formations. Three types of reaction were noted. One consisted of small areas of complete fibrosis surrounded by lymphocytes and large mononuclear cells. Another lesion consisted of areas of fibroblastic reaction with or without a slight amount of early necrosis and with peripherally and centrally located giant cells which appeared to be surrounding clear spaces and debris. Other lesions consisted of a vesicular body with peripherally or centrally arranged nuclear masses and surrounded by a clear space excepting on one side. Just outside the clear space a fibroblastic proliferation was taking place. Lymphocytes and occasional polymorphonuclear leucocytes surrounded the outer fibrous area.

Skin. The skin showed a similar reaction with degeneration and giant cell formation as prominent features.

Bone marrow. The femur marrow showed hyperplasia of all elements. Numerous nodules similar to those already described were scattered throughout the sections. However, necrosis was a prominent feature in this area.

Special stains for bacteria including acid fast organisms were repeatedly negative.

DISCUSSION

The sequence of events with reference to the widespread echinococcus infection is fairly difficult if not impossible to follow in this case. The two liver cysts were healed and appeared to be of much longer duration than the lesion in the spleen. The cyst in the spleen showed both degenerative changes and

the presence of numerous daughter cysts, and appeared to be of more recent origin.

In his discussion of death and degeneration of cysts, Dew[5] states that caseation necrosis occurs with the production of white, yellowish or gray material containing fatty and granular debris and remains of the parasite. He says this degenerative change has been mistaken, when well advanced, for a caseating tuberculous focus. Heller[2] applied the term echinococcosis or hydatidization to widespread dissemination of the disease and gives four modes by which this dissemination may occur: widespread embolic implantation of the embryos; secondary implantation of scolices, or brood capsules, in a serous cavity or different organs; propagation into adjacent organs by exogenous budding and direct extension; or infection by ingestion, implantation, or inhalation of fertile cysts or viable scolices. He doubts whether infection by inhalation ever occurs though he believes it is possible.

Le Nouene,[6] in reporting twenty-six cases of hydatid pseudotuberculosis of the peritoneum, quoted Deve's histologic description of the tubercle-like lesions formed in the peritoneum. Deve's study, obtained from both human and experimental material, showed three types of lesions. One consisted of a central area filled with debris, with or without hooklets, bile pigment, cholesterin, or lime salts, surrounded by epithelioid cells mingled with giant cells. A second reaction consisted of a fibroid organization of the scolex in which all elements had disappeared leaving only a small connective tissue nodule with no characteristics. The third type of lesion consisted of the active vesicle surrounded by a zone of epithelioid and giant cells. Le Nouene[6] states that the second type of reaction has only been observed in experimental production of echinococcosis.

It is believed that the lesions found in this case resulted from rupture of the splenic cyst into the substance of the spleen with spread by the blood stream, lymphatics, and direct extension. The histologic study showed early lesions with degenerative changes and fibroid organization of the scolex.

SUMMARY

1. A case of disseminated echinococcosis is presented.

2. The spread of the disease is believed to be both embolic and by direct extension to adjacent tissue and lymph nodes.

3. The histologic picture represents three types of lesions, that of foreign body reaction to debris, fibrosis with tubercle formation, and a fibroblastic reaction around centrally arranged nuclear masses.

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EDITORIAL

The Need For A Standardized Hematologic Nomenclature

Present day hematologic nomenclature is confusing, and ought to be revised. To prove this statement the following illustrations are only a few of those which could be given. The familiar *monocyte* has about twenty synonyms—*endothelial leucocyte, transitional, endotheliocyte, histiocyte, epithelioid cell* and so forth. Terms such as *erythrogonia, hematocyte,* and *lymphoidocyte* have to be defined anew each time they are used, because they indicate different cells to different authors. *Malignant* neutropenia, first described in 1922, is now referred to by some fifteen different names. *Aleukemic leukemia* also is known by at least ten different names, some lately introduced.

The recent publication of two atlases of hematology[1,2]—each advocating a terminology of its own—serves but to complicate the situation. These atlases are written by teachers of graduate and undergraduate courses in Medicine; both have been recommended for student use. Their readers are required to familiarize themselves with new terminologies, at least one of which cannot survive.

The question arises as to whether or not a satisfactory nomenclature can be worked out. Many agree that it can and that the project is a worthy one, even if additions and revisions are made necessary by new discoveries. At least certain basic definitions and terms could be made and many useless synonyms discarded. That by itself would make the effort worth while.

What machinery can be set up to achieve this purpose? Only a few suggestions can be offered here; the details should be worked out by a group appointed for that purpose.

The A. S. C. P. should initiate the movement for the suggested revision. As a preliminary step, a committee could be appointed to inquire into the feasibility of the project. This

committee should be authorized to collaborate freely with interested members of other national societies. This would ensure that the final draft of the committee would reflect the consensus of internists, hematologists, histologists and pathologists. The findings of the committee should be published in a widely circulated medical journal and in pamphlet form. Sale of the latter would help to defray some of the expenses incurred, and the rest could be met by grants from this and other societies.

HAROLD GORDON

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THE TECHNICAL SUPPLEMENT

Perhaps of all who are engaged in one or more of the varied phases of medical practice, the Clinical Pathologist observes the most striking illustrations of the axiom that medicine is more or less constantly in a state of flux.

It is true that pathological concepts suffer change slowly and even reluctantly. Diagnosis, based upon concepts of the mechanism and results of disease, also advances with a somewhat measured step. Therapeutics sometimes seems like a troubled ocean with waves of enthusiasm rising to mountain height not infrequently followed by sinking valleys of scepticism and discouragement so that time and time alone can determine how the picture shall eventually be drawn. But the field of laboratory medicine may suffer abrupt and striking changes with relatively great rapidity as the accepted procedure of today is dethroned by the publication of tomorrow.

Clinical pathologists, perforce, become accustomed to this and realize the urgent necessity for close contact with the literature of their field. Mere familiarity with the literature, nor even with the last textbook, however, is not enough. For text books take time to write, to print and to put into circulation so

that the technic accepted when the manuscript was written may have been effectually modified by the time the bookis in circulation.

The medicine of the future may well be said to be written in the medical journals of today, and this is even more true of laboratory procedures and their utilization, so that publications in this field must be closely watched by the laboratory worker lest he fall behind in the march of progress.

The difficulty, however, is to evaluate them. Is this new method *really* better than the old? Was there not a method published somewhere which would be peculiarly applicable to this particular problem? Does this method sound worth a trial, or what has been the experience of others with that one? Would it not be a good idea to survey the field and bring these other procedures up to date?

Perhaps it would—if there were time, if enough help were available, if library facilities were handy. If—!

Unfortunately, not all the current literature is equally accessible and, moreover, many an excellent technic is lost in the mists of obscurity simply because, for some reason or another, it failed to attract sufficient attention.

It was from such considerations as these that the idea of a Technical Supplement to the Journal was born. Intended primarily as an aid to the laboratory worker, its purposes are:

1. To present in full detail new methods, or modifications of old ones, which the test of time and trial had suggested as worthy of trial, at least, if not adoption.

2. To present from time to time succinct epitomes of specialized procedures selected by competent groups as embodying accepted methods.

3. To call attention to methods culled from the literature as worthy of study and, by the experience of those who tried them, to arrive at their proper evaluation.

4. To call attention, also, to the little "tricks of the trade," so to speak, which may come in handy now and then.

5. To serve as a forum for the elaboration of technical difficulties and their solution.

To make this material readily accessible as a laboratory work-

table reference, the Technical Supplement is separately issued in a form easily kept in a standard binder and may be subscribed for separately so that, in addition to the laboratory copy, one may be available to every worker desirous of "keeping up with the times."

There is ample evidence that the Supplement is serving the purposes for which it is designed, but it cannot achieve them to the fullest degree without the active interest and cooperation of all in whose interests it came into being.

Have you, who read and perhaps made use of its contents utilized it as an addition to your laboratory reference file? Has it occurred to you that your technicians might find use for a personal file of the Supplement issues or, indeed, that this would be desirable?

From your experience in evading or solving the trials and tribulations common to all laboratory workers have you made—or contemplated—any contribution to its pages?

Perhaps it may be only a minor modification which you regard as too trivial for a formal "paper" and yet one which has more than proved its worth.

The pages of the Supplement may be just the place for it and the thing itself just what someone else is looking for.

One of your technicians may have some little trouble with the preparation of tissues. Have you called her attention to the contributions on this subject which have appeared in the Supplement and suggested that a file of its issues for her own use would be a good—and cheap—investment?

As some one once said, "it isn't the books you have in your library, it is the books you use!"

Do you use the Supplement; has it occurred to you how useful it can be—not once in a while, but frequently?

If, perhaps, you wish that some specific thing had been presented, why not suggest that as a subject for consideration by the Editorial Board? If you know of a still better laboratory trick than one reported, why not write it up for the Supplement?

It can only be what *you* help to make it. You can easily make it what you will if every member of the American Society

of Clinical Pathologists regards it as peculiarly his own, conceived for him and to be carried on by him.

When *you*, by your contributions, queries, and suggestions; by your use of the Supplement; by calling the attention of your technicians to its utility, assist in its development, then, and only then, will the Technical Supplement achieve in full the goal set for it by the Board of Registry and the American Society of Clinical Pathologists whose united endeavors brought it into existence.

As a matter of fact, one of the main purposes the Technical Supplement was intended to serve was as a medium whereby the technician might easily and with a minimum expenditure of effort and expense, keep in touch with the interesting and important developments in the laboratory field. It was for this reason that the Society assumed the not inconsiderable expense of publishing the Supplement in very cheap form so that the technician, at little expense, might have the material as a permanent and accessible reference. For it was felt that to spend $1.50 a year on that which obviously aided in the efficient performance of their work would not be too much of a burden.

Somewhat strangely, however, this particular phase of the Supplement seems to have been neglected. Either Laboratory Directors have failed to emphasize and encourage it; or else technicians themselves have failed to appreicate the advantage and necessity of keeping in touch with the changes in their own field. For, unless there is an appreciation of this necessity, unless Laboratory Directors and their personnel give clear evidence of their interest, best evidenced by subscribing to the Supplement, it is questionable whether the Society can or should be asked to carry the financial burden of its maintenance indefinitely.

Richard Herman Jaffé
1888–1937

Richard Herman Jaffé was born in Vienna, June 13, 1888. After completing a humanistic gymnasium, he entered the Medical School of the University of Vienna. After graduation in 1912, a period of study followed in various laboratories (with Abderhalden in Halle, Stoerck in Vienna, and at the Institute for Tropical Medicine at Hamburg). In 1914 he returned to Vienna and became associated with Paltauf at the Sero-Therapeutic Institute. Paltauf had a profound influence on the entire future scientific career of Jaffé.

During the war, Jaffé served as Prosektor at a large military Hospital in Vienna (First Garnisons Spital) which offered him a great wealth of pathologic material. In 1922 he was made Privatdozent at the Medical School of the University of Vienna.

About this time, Dr. Charles S. Bacon, Professor of Obstetrics at the University of Illinois and President of the Medical Staff of the Grant Hospital, Chicago, visited Vienna trying to find a pathologist for the newly equipped department of pathologic laboratories of the Grant Hospital. He induced Jaffé to accept this position.

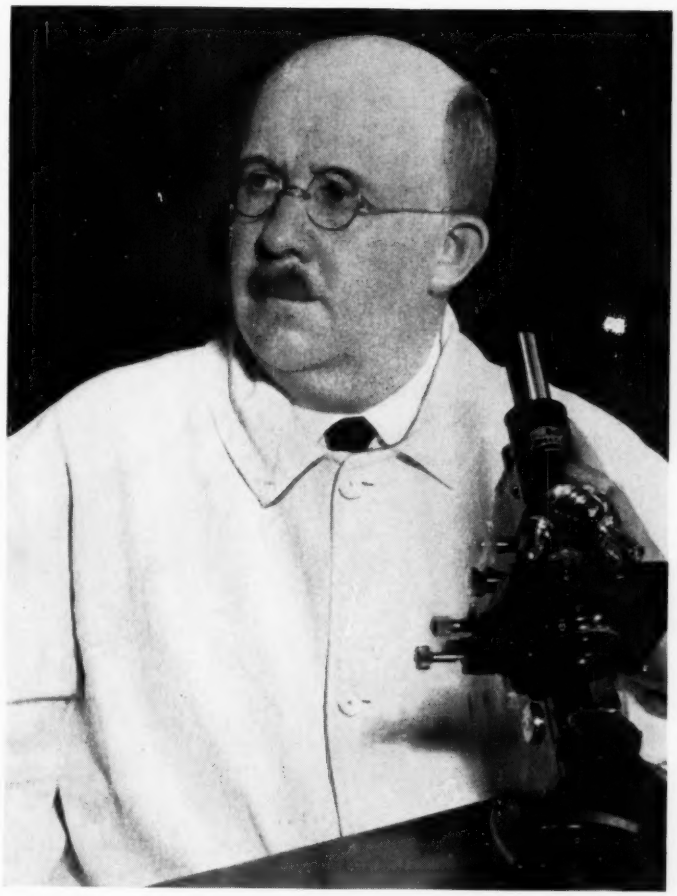
In addition to the position at the Grant Hospital, Jaffé was appointed to the staff of the Department of Pathology and Bacteriology of the University of Illinois College of Medicine.

When provisions were made for a proper salary for the position of pathologist of the Cook County Hospital, Jaffé was appointed Director of the Laboratories in 1928. At that time began the most fruitful period of his activities in Chicago. The pathological conferences on Thursday mornings became a recognized institution, the high spot of medical Chicago, attracting internes and the entire medical staff of the Cook County Hospital, medical practitioners of the whole city and considerable numbers of out of town visitors.

Jaffé was a born teacher; he taught with a thoroughness and a devotion that came from a love of teaching. This ability, together with a mastery of the whole field of pathologic anatomy resulted in clinics and lectures that were the delight of many thousands of physicians. Jaffé was truly a teacher of physicians. His influence extended far beyond Chicago through lectures in many cities, through his publications and through the activities of his students. He conducted the Tumor Seminar of the American Society of Clinical Pathologists in Kansas City in 1936. Those who participated in it will never forget the excellence of his presentations.

His devotion to the work kept Jaffé from heeding the advice and warnings of his friends and physicians when they became aware of his serious illness. He conducted the weekly pathological conference on Thursday, December 16, and the monthly pediatric pathological conference on the following day. He died suddenly on the evening of that same Friday, December 17, 1937. In Richard Herman Jaffé Clinical pathologists have lost a great friend and leader.

ISRAEL DAVIDSOHN



Richard Herman Jaffé
1888–1937

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OBITUARIES

Dr. Charles August Bentz was born in 1879 in Buffalo, New York. He graduated from the University of Buffalo School of Medicine in 1902. He was a member of the American Association of Pathologists and Bacteriologists, the Society of American Bacteriologists and a member of the American Society of Clinical Pathologists since 1922. For many years he served as an officer of the Medical Society of the County of Erie. He was Director of the Division of Communicable Diseases, Superintendent of Laboratories and Deputy Commissioner of the City Department of Health. At various times he was on the staffs of the J. N. Adams Memorial Hospital, Perrysburg Hospital, Buffalo Eye and Ear Infirmary, Buffalo Hospital of the Sisters of Charity; Memorial Hospital and St. Mary's Maternity Hospital. Dr. Bentz was especially gifted as a teacher and endeared himself to the student body in the School of Medicine

of the University of Buffalo during his service there as Assistant Professor of Medicine. Dr. Bentz died July 25th, 1937 of endocarditis at the age of 58.

Dr. Solomon Leon Cherry, of Clarksburg, West Virginia, died October 21, 1937, of hypertensive heart disease at the age of 50.

A native of Russia, Dr. Cherry came to America in 1890 with his parents at the age of three years and attended Philadelphia and Baltimore public schools. He was graduated at Baltimore City Medical College in 1904 and continued his work at the University of Maryland until 1908. Until a year before his death Dr. Cherry was in charge of the laboratories at St. Mary's Hospital, having come there in 1918 from Mt. Sinai Hospital, Philadelphia.

His record in medicine was distinguished. He served the Harrison County Medical Society as president and secretary, was a member of the Catholic Hospital Association, the West Virginia State Medical Association, the Southern Medical Association, was a fellow of the American College of Physicians and a member of the American Society of Clinical Pathologists. He was captain in the Medical Corps in 1918 and 1919 and went to France as officer-in-charge of the laboratory in Evacuation Hospital No. 24.

He served as bacteriologist for the Clarksburg City Health Department, as a laboratory consultant for the United States Veterans' Bureau and was secretary of the Medical Advisory Board of St. Mary's Hospital, Clarksburg.

Surviving Dr. Cherry are his wife and two sons.

Dr. Daniel Francis Daley was born in Kingston, Pennsylvania in 1887. He graduated from the Jefferson Medical College of Philadelphia in 1915. He was a fellow of the American College of Physicians and a member of the American Society of Clinical Pathologists since 1924. He served the Mercy Hospital, Wilkes-Barre, in various capacities. Dr. Daley died of heart disease on April 24, 1937 at the age of 50.

Dr. Thomas Tipton Walker was born in Atlanta, Georgia, March 11, 1904. He graduated from Emory University in 1924 and received a Master's Degree from the University of North Carolina in 1925. He entered Harvard Medical College and in 1928 a scholarship took him to London, where he worked at St. Thomas' Hospital under Sir Cuthburt Wallace.

After graduation from Harvard Medical College he held a residency in pathology at the Boston City Hospital and then took postgraduate work at Eppendorfer Krankenhaus and at the Frankfurt Stadische Krankenhaus. He then accepted a position as pathologist at the Duke Hospital, Durham, North Carolina, and became instuctor in pathology at Duke University. In 1932 he was appointed director of laboratories at the House of the Good Samaritan and Mercy Hospitals, Watertown, New York, and later consulting pathologist

at the Jefferson County Sanatorium at Watertown. These positions he fulfilled most efficiently and rendered this community the highest type of pathological service.

He was a diplomate of the National Board of Medical Examiners and also of the American Board of Pathology; an officer of the Jefferson County Medical Society; a member of the New York State Medical Society; the American Medical Association; the American Association of Pathologists and Bacteriologists; the New York State Association of Public Health Laboratories; the Pathological Society of Eastern New York and an associate of the American College of Physicians. He was a member of the American Society of Clinical Pathologists since 1934.

Dr. Walker died November 13, 1937, at the age of 33. He is survived by his widow, Lillie Cutlar Walker.

Dr. Pearl Caleb West was born in 1871. She attended the Saginaw Valley (Michigan) Medical College in 1902 and Columbia University College of Medicine, New York, in 1911. Dr. West served during the World War. She was pathologist to the Northern State Hospital. Dr. West was a member of the American Society of Clinical Pathologists. She died on December 31, 1937, at the age of 66.

Dr. Courtland Yardley White was born in 1872. He received his medical degree from the University of Pennsylvania in 1895. After serving his internship at the Philadelphia General Hospital he studied in Vienna and Leipzig. He early became associated with the Pepper Laboratories and Phipps Institute of the University of Pennsylvania, where he was instructor in clinical medicine from 1899 to 1904, lecturer and demonstrator in morbid anatomy in the Veterinary Department, and later associate professor of bacteriology at Medical Chirurgical College. He was director of the pathologic laboratories of the Episcopal Hospital from 1908 to 1938; director of the City of Philadelphia Laboratory of Bacteriology from 1910 to 1938; and at various times on the staffs of the Children's Hospital, Kensington Women's Hospital, St. Joseph's Hospital and Jewish Hospital. He was a fellow of the Philadelphia College of Physicians, a member of his county and state medical societies, the Pathological Society of Philadelphia, the Academy of Natural Sciences, and of the Association of Pathologists and Bacteriologists. Dr. White was a member of the American Society of Clinical Pathologists since 1926. He was a former president of the Sydenham Coterie, a member of the Union League, and a member of the Penn Athletic Club.

Dr. White, a prominent figure in medicine in the city of Philadelphia for many years, was also a well known sportsman. He died of arteriosclerosis on January 14, 1938 at the age of 65, and is survived by his wife, Emily Heroy Sherwood, and three sons, Courtland Yardley III, William S., and Henry.

NEWS AND NOTICES

Technicians' Institute

The Technicians' Institute held in Philadelphia on April 11th, 12th and 13th by Temple University School of Medicine, under the direction of Dr. John A. Kolmer and with the coöperation of the Pennsylvania Society of Medical Technologists, was so successful from the standpoint of attendance, excellence of program and arrangements as to indicate that conventions of this kind held at intervals will be of great service and benefit to medical laboratory technicians.

The program, consisting of lectures, addresses at the evening dinner session and laboratory demonstrations with exhibits, was published in the March issue of this *Journal*. A noteworthy feature was the privilege afforded technicians for submitting questions in writing which (totalling 84) were read and answered by the Faculty of the Institute at the evening dinner session at which 218 were present. Dr. Robert A. Vonderlehr was unable to be present at this session and Surgeon J. F. Mahoney, Director of the Venereal Disease Research Laboratory of the U. S. Public Health Service, gave the address on "The Rôle of the Technician in the Campaign Against Syphilis."

The Institute was open to graduate and student technicians of Pennsylvania, New Jersey and Delaware and programs were sent to all technicians registered with the Registry of Medical Technologists of the American Society of Clinical Pathologists, as well as to the superintendents of all hospitals in these three states. The attendance was beyond expectations and totalled 304, including 4 physicians, 252 graduate and 48 student technicians distributed as follows:

Pennsylvania	217
New Jersey	65
Delaware	16
New York	2
Washington, D. C.	2
Ohio	1
Indiana	1

* * * *

The following were elected as officers of the Michigan Pathological Society for 1938:

President: R. C. Wanstrom, M.D., Ann Arbor.
President-Elect: V. W. Lohr, M.D., Saginaw.
Secretary-Treasurer: W. L. Brosius, M.D., Detroit.

Councillors: G. L. Bond, M.D., Grand Rapids; D. A. Humphrey, M.D., Battle Creek.

This Society, composed of twenty-six active and nine Associate members, meets five times a year. The meetings are frequently in the form of symposia and interesting exhibits and cases are also presented. During 1937, for example, the following subjects were presented: Pathology of The Male Generative System and the Urinary Bladder; Endometrial Pathology; Pathology of The Liver; Degenerative Pathology of the Nervous System; and Criminological Pathology.

The first meeting of 1938 was devoted to Tumors Exhibiting Hormonal Activity.

* * * *

The 67th Annual Meeting of the American Public Health Association will be held in Kansas City, Missouri, October 25–28, 1938.

BOOK REVIEWS

Snow on Cholera. A Reprint of Two Papers By JOHN SNOW, M.D. together with a Biographical Memoir by B. W. RICHARDSON, M.D. and an Introduction by WADE HAMPTON FROST, M.D., Professor of Epidemiology, The Johns Hopkins School of Hygiene and Public Health. Cloth, 250 pp. $2.50. The Commonwealth Fund, New York.

This is a book of outstanding interest. When it is recalled that the cause of cholera remained unknown until a quarter of a century after Dr. Snow's death, the remarkable clarity of his concept of the disease, the insight with which he divined the mechanism of its spread and propagation, and the accuracy with which he interpreted the data he gathered so painstakingly and with such purposeful intelligence, all combine to make his contributions of epochal importance in the history of medicine.

As Dr. Frost comments in his introduction: "It is not easy, when divergent theories are presented, to distinguish immediately between those which are sound and those which are merely plausible. Therefore it is instructive to turn back to arguments which have been tested by the subsequent course of events; to cultivate discrimination by the study of those which the advance of definite knowledge has confirmed. A nearly perfect model is John Snow's analysis of the epidemiology of cholera."

Outside of its value as an historical landmark in medicine, this book can be read with pleasure for its intrinsic interest, its picture of Old World Sanitation, and its vivid and living reproduction of its time. For John Snow was not only a physician and epidemiologist of mark, he was, as these papers show, also a writer of skill.

This is a book which can be read repeatedly and with continuing interest. It is a book which can be read with enjoyment as well as profit and owned with pleasure. It can be recommended without reserve.

Recent Advances In Pathology. By GEOFFREY HADFIELD, M. D., Professor of pathology, University of London, and LAWRENCE P. GARROD, Professor of Bacteriology, University of London. Ed. 3, 420 pp., 65 figures. $5.00 P. Blakiston's Son & Co. Inc., Philadelphia.

In the four years which have elapsed since the last edition of this member of the "Recent Advances" series, many changes have occurred necessitating extensive revision to bring the volume up to date.

New chapters on "Resistance To Infection" and "Reticulosis And The Reticulo-Sarcomata" have been added; extensive alterations have been made

in the Chapters on Cancer and on Deficiency Diseases; the sections on experimental cancer research have been largely rewritten; and extensive revisions and alterations are found throughout the book.

As before, this book furnishes an excellent review of present concepts of pathology.

The Thyroid And Its Diseases. Being An Account Based In Large Measure On The Experience Gained In The Thyroid Clinic Of The Massachusetts General Hospital. By J. H. MEANS, M.D., Jackson Professor of Clinical Medicine, Harvard University, And Chief Of The Medical Services, Massachusetts General Hospital. Cloth. 602 pp., 73 figures. $6. J. B. Lippincott Co., Philadelphia, Pa.

While, as stated in the Preface, this book is not intended to be encyclopedic, it constitutes, nevertheless, an exceedingly comprehensive survey of the diseases of the thyroid gland.

The work is based upon the extensive series of cases seen in the Thyroid Clinic of The Massachusetts Hospital since its beginning in 1913 and presents the thoroughly considered experience of the clinic personnel. This volume is, therefore, as it might be expected to be, a contribution of outstanding value to physician, surgeon and pathologist alike.

The particular value of the book depends, not alone upon the completeness of the category of the diseases of the thyroid which it discusses, nor upon the volume of clinical material utilized, nor even upon an exceptionally thorough follow-up, but rather upon the care with which this extensive experience has been critically digested and evaluated. This is a valuable contribution to the literature on thyroid diseases.

The Conquest of Cholera. By J. S. CHAMBERS, M.D., Professor of Hygiene and Public Health, and Director of Student Health Service, University of Kentucky. Cloth, 366 pp., 40 illustrations. $4.75. The MacMillan Company, New York.

This is, indeed, an interesting book in which, step by step, the author tells the story of Asiatic Cholera in the United States from its initial outbreak in 1832 until its ultimate control and conquest sixty years later.

Dr. Chambers has constructed not only a tale of outstanding interest, but one which is authoritative in every respect as it is based upon a thorough and extensive study, not only of official reports, but upon contemporary news accounts, public records and personal accounts.

Thanks to the progress made in medicine and sanitary science, present generations have little or no concept of disease in epidemic form as depicted in the graphic pages of this book. Of particular interest is the gradual emergence of an understanding of the nature, cause, method of transmission of the disease, the slow transmission of thought from miasms to microbes.

This book is more than a story of cholera, it is, in effect, a chapter in the

medical and general history of the United States which can be read with pleasure as well as profit.

A Textbook of Clinical Pathology. Edited by R. R. KRACKE. Cloth, 577 pp., 201 illustrations, 31 colored plates. $6.00. William Wood & Co., Baltimore.

This book, prepared by twelve contributors under the Editorship of Dr. Kracke, is an outgrowth of the deservedly popular "Laboratory Diagnosis" of Bass and Johns and represents the development of an idea conceived by the late Foster M. Johns.

It appears from the preface that the volume, which is stated not to be a manual of laboratory technic but one stressing interpretation, is addressed particularly to the medical student and physician. The concept is born out by the opening chapter (The Physician's Laboratory) and by the organization, contents, and tone of the volume as a whole.

This is a particularly difficult type of book to compile. If restricted to those matters feasible and properly to be carried out in an office laboratory, the book obviously cannot serve as a satisfactory reference text for the well trained technician and the clinical pathologist. If satisfactory for these readers, it must inevitably include matter and procedures far beyond the scope of an office laboratory.

It is, of course, apparent that neither an extensive reference library nor an extensively equipped laboratory can make of the physician a clinical pathologist nor a technician of his office girl.

The primary problem in the construction of a book of this kind is, then, what shall be selected for inclusion and how extensively shall these subjects be discussed. And the problem is by no means easy of solution. For the procedures properly belonging to the office laboratory are necessarily restricted, nor can their clinical interpretation be satisfactorily discussed with undue brevity. It may be questioned, perhaps, whether such disorders as leukemia, leukopenia, and infectious mononucleosis can be thoroughly discussed from both clinical and laboratory viewpoints in thirteen pages of large type. Or whether the interpretation of urinalysis can be compressed into seven pages, or the examination of sputum thoroughly covered in eight pages.

Certainly, complement-fixation tests have no place in the physician's laboratory nor can even the simpler precipitation tests be safely left to the hands of those not basically well trained in serological principles.

Few will agree that safe typing serums can be secured simply by collecting blood from healthy group A and B males. Coca long ago emphasized that typing serums *must* be of high titer and must be selected on that basis.

However, books of this sort serve a useful purpose in calling the attention of physicians to the variety of laboratory procedures applicable to the study of disease; in emphasizing the importance of their technical minutia; and thus, indirectly, suggesting, perhaps, the advisability of referring the more compli-

cated procedures and those requiring skill and particularly experience for their satisfactory performance—as well as their accurate interpretation—to those properly prepared to handle them

The Biology of Pneumococcus. The Bacteriological, Biochemical, and Immunological Characters and Activities of Diplococcus Pneumoniae. By BENJAMIN WHITE, PH.D. with the collaboration of ELLIOTT STERLING ROBINSON, M.D., PH.D. AND LAVERNE ALAMN BARNES, PH.D. Cloth, 779 pp. $4.50. The Commonwealth Fund, New York.

This is a timely volume the purpose of which, as set forth in the Preface is "to sort out the accumulation of more than fifty years, to convert old specie into modern currency, and finally to set a value on the whole store."

In view of the present and increasing general interest in pneumonia this volume should prove of great interest to physicians, public health workers, and clinical pathologists as bringing under one cover all that is known of the pneumococcus. It may be accepted as a comprehensive, critical and authoritative evaluation of all the information available concerning the pneumococcus and, as such, should become an outstanding reference text for years to come.

The Diary of A Surgeon In The Year 1751–1752. By JOHN KNYVETON, Licentiate of the Society of Apothecaries; Doctor of Medicine of The University of Aberdeen; Teacher of Midwifery and Man-Midwife In Infirmary Hall; Surgeon's Mate H.M.S. Lancaster. Edited and transcribed by ERNEST GRAY. Cloth, 322 pp.; 7 illustrations. $2.50. D. Appleton-Century Co., New York.

The jacket of this book says: "This is one of those intriguing books which defies classification. It can be enjoyed as a vivid reconstruction of old medical practice, it can be read as a tale of high adventure on the sea, or it can be savored as a delightful piece of writing with a distinctive Pepysian flavor. Whatever it is, it makes thrilling reading—an unusual treasure of a book which does more to throw light on past days than reams of histories."

It is permissible sometimes to use the proverbial grain of salt with publisher's announcements—but not with this book. It more than lives up to what is said about it above. It is more than a delightful and intriguing book, it is one which is only laid down with reluctance. A book to read, reread and read again, it is not a book to be lent, for once read it will only be returned under protest.

Here is a book that can be ordered with no fear of ever regretting the impulse, for it will undoubtedly become a classic. If you but open it you will want to own it and if you do you will place it on your own particular favorite shelf among the books you read again and again with increasing enjoyment.

The Treatment of Clinical and Laboratory Data, An Introduction To Statistical Ideas and Methods for Medical and Dental Workers. By DONALD MAINLAND, M.B., D.Sc., Professor of Anatomy, Dalhausie University, Halifax,

Nova Scotia, Canada. Cloth, 340 pp., 23 text figures. Oliver and Boyd, Edinburgh.

The purpose of this book is to present to the clinician, dentist and investigator the methods applicable to the statistical evaluation of clinical or research data.

As the author says if statistical data is often in disrepute it is partly because of confusion between statistical data and statistical methods, and partly because of ignorance of the basis of the methods and of their legitimate use.

It is the purpose of this book to dispel this confusion and remedy this ignorance and as a clear and understandable exposition this book may be highly recommended.

The Compleat Pediatrician, Practical, Diagnostic, Therapeutic and Preventive Pediatrics. By WILBURT C. DAVISON, M.A., D.Sc., M.D., Professor of Pediatrics, Duke University School of Medicine, and Pediatrician Duck Hospital. Cloth Ed. 2., $3.75. Duke University Press.

It is not surprising that this book has reached a second edition for, as was emphasized in reviews of the first edition, it is an exceedingly practical contribution to pediatric literature.

This second edition is even a better book than the first in that it has been extensively revised and almost entirely rewritten as well as reorganized so that its contents are more accessible.

It is presented to the reader, not as a textbook on pediatrics but as a digest of pediatrics and, particularly, as a ready reminder.

Perhaps in no field of medical practice may the problems of diagnosis be more acute than in pediatrics, often because the physician must largely rely, first, upon what his own observations can elicit, and second, upon the extent to which his recollection and experience familiarize him with the diagnostic possibilities. As the author remarks in his preface, this book (nor any other, for that matter) will not do his thinking for him but it well serves to jog his memory.

As before, the plan is more or less reminiscent of a thesaurus. Under each main presenting symptom are found cross references to various conditions in which it may occur, together with additional signs and symptoms these present and so on. In the reorganization of the book the symptoms and diseases have been wisely gathered in seven chapters on the basis of the anatomical system chiefly involved. The book is easy to use, contains a tremendous amount of information and evinces not only an extensive practical experience, but a comprehensive familiarity with pediatric literature.

To the student, intern, practitioner, pediatrician alike this volume should prove exceedingly useful. The pathologist will find it valuable as a reference to the interpretation of laboratory findings in children.

Practical Bacteriology, Haematology and Animal Parasitology. By E. R. STITT, M.D., Sc.D., L.L.D., Rear Admiral, Medical Corps and Surgeon General,

U. S. Navy, Retired, etc., PAUL W. CLOUGH, M.D., Chief of Diagnostic Clinic, Johns Hopkins Hospital, and MILDRED C. CLOUGH, M.D., Formerly Fellow in Bacteriology and Instructor in Medicine, Johns Hopkins University. Cloth, Ed. 9, 961 pp. 208 figures, $7.00. P. Blakiston's Son & Co., Philadelphia.

This is a volume which requires no introduction for copies of Stitt's book—showing the honorable scars of long and continuous use—are probably to be encountered on more laboratory work tables and more working bookshelves than any other book of its kind. So long has it been since the appearance of the last edition (over ten years) that this new edition will be more than welcomed by the innumerable users of this text.

This new text presents itself in a new guise, increased not only in size but containing one hundred and twenty-four more pages. The entire text has been reorganized and rewritten to include a large amount of new material reflecting the changes and advances of the last ten years so that the volume may be regarded as up to date.

It is unnecessary to describe this book nor even to say that if one were restricted to only one book of this kind, Stitt's volume might well be the one, or to add that no matter how extensive the working or reference laboratory may be, no mistake will be made in adding the new edition of Stitt to the list.

THE EARLY DIAGNOSIS OF ACUTE AND LATENT PLUMBISM*

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Information gained from the evaluation of laboratory findings is only of importance when it aids in establishing diagnosis, in choosing therapeutic agents, or in indicating the probable prognosis. In order to accomplish this end it is necessary that such findings furnish information far enough in advance of clinical signs and symptoms to enable the introduction of therapeutic measures in time to avoid, or lessen the severity of a crisis. This is of even greater importance when dealing with the introduction and absorption into the body of a substance which in excessive amounts may cause an acute toxic condition, or produce such an effect at a later date by its accumulation in the body and its subsequent release due to a lowering of the normal tolerance and some uncontrollable change in the equilibrium of physiological and biological factors. Among such toxic agents lead occupies a prominent position.

Although no single sign or symptom may be considered pathognomonic of plumbism, certain diagnostic features have been attributed to acute and chronic lead poisoning. These have been classified and discussed in detail by Jones[1], Harris[2], Shie[3], Aub and his associates[44], Lowy and Levinson[4], Vogt and McKhann[5], Mitchell[6], Johnson[7], and Kehoe[8]. In order to facilitate the tabulation of our data we have grouped them in the manner shown in table 1.

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TABLE 1

Clinical signs and symptoms of plumbism

GROUP I SUGGESTIVE EVIDENCE OF LEAD ABSORPTION	GROUP II SUGGESTIVE EVIDENCE OF INCIPIENT INTOXICATION AND INACTIVE OR ARRESTED PLUMBISM	GROUP III SUGGESTIVE EVIDENCE OF DEFINITE, ADVANCED, AND ACTIVE PLUMBISM WITH ACUTE MANIFESTATIONS
General symptoms		
Patient becomes easily flustered, moody, restless and excitable	Pallor	Anemia
	Jaundice	Inanition
	Slight lead line	Lead line
	Arthralgia	Arthralgia
	Slight inanition	Jaundice
General feeling of malaise	Fatigued easily	General weakness
	Hypotension to Normal	Hypertension
	Slight pyrexia	Pain in chest
		Wrist drop
		Foot drop
Digestive system		
Persistent metallic taste	Metallic taste	Metallic taste
	Coated tongue	Coated tongue
Slight anorexia	Anorexia	Anorexia
Slight constipation	Constipation	Marked constipation
	Slight abdominal colic	Paroxysmal colic
		Nausea and Emesis
		Rigid abdomen
		Blood in stool
Nervous system		
Irritability	Slight frontal headache	Severe frontal headache
Uncoöperativeness	Slight tremors to Parkinsonian Syndrome	Tremors
		Confusion
	Slight ataxia	Ataxia
	Insomnia	Insomnia
	Palpitation	Convulsions
	Increased reflexes	Fibrillary twitchings
	Increased irritability	Neuritis
	Eye grounds may show choking of optic discs	Visual disturbances
		Encephalitis
		Hallucinations
		Coma
		Paralysis
		Cerebral palsy

TABLE 1—*Concluded*

GROUP I SUGGESTIVE EVIDENCE OF LEAD ABSORPTION	GROUP II SUGGESTIVE EVIDENCE OF INCIPIENT INTOXICATION AND INACTIVE OR ARRESTED PLUMBISM	GROUP III SUGGESTIVE EVIDENCE OF DEFINITE, ADVANCED, AND ACTIVE PLUMBISM WITH ACUTE MANIFESTATIONS
	Renal symptoms	
Lead which fluctuates between normal limits and a very slight rise	Trace of albumin and few granular casts in urine Lead which fluctuates between normal limits and a positive rise	Toxic nephrosis Albuminuria Casts in urine Hematoporphyrinuria Hematuria Positive but fluctuating lead findings

It should be remembered that all of these symptoms will never be found in any single case and frequently a patient is presented for observation or treatment whose past history to lead exposure would cause one to expect symptoms conforming to Groups II or III when only those of Group I can be demonstrated.

Many lesions have been reported associated with the above symptoms of lead poisoning by the previously named investigators, as well as Taylor and Schram[9] and Vigdortchik[10], the most common of which are: Arteriosclerosis, ulcers of the stomach and intestine, contracted small intestine, tubular and chronic interstitial nephritis, hemorrhages and exudates of the retina, neuro-retinitis, chronic nephritis (particularly in children), malignant hypertensive neuroretinitis, hypertensive encephalopathy, hypertonia, neuronophagia in cerebral sections, essential hypertension.

Carlson[11] showed that the symptoms of chronic arsenic poisoning resemble those of plumbism, and Lanza[12] concluded that many cases of mild lead poisoning are diagnosed as chronic appendicitis and even as gall bladder disease, with all too frequent surgical intervention. We have known cases of mild lead poisoning to be confused with intracranial and cord tumors. It has been shown by the majority of these investigators that many workers continually exposed to lead fail to exhibit clinical evidence of plumbism. This individual variance in susceptibility

to lead intoxication is also shown in subjects injected with lead. As little as 75 mgs. of lead (intravenously injected as a colloidal lead manganese phosphate preparation) has been known to cause the development of severe symptoms in some individuals while in a few others signs and symptoms of lead toxicosis did not appear after the introduction of 900 mgs. of the same preparation. Of course this variance in susceptibility is influenced by many pathological conditions among which are anemia, anoxemia, malnutrition, vitamin deficiencies, blood dyscrasia, and diseases of the kidney.

Lead poisoning in infants and children is less difficult to diagnose than latent or incipient plumbism in the adult, although, as Cushing[13] has shown, such late manifestations as lead line, colic, and wrist drop are usually absent. Aub, Robb, and Rossmeisl[14] found that lead stored in the bones is present in a higher concentration in the trabeculae than in the cortex, while in children the highest concentration occurred in the areas where the calcium is being most rapidly deposited. This finding was confirmed by Kasahara and Nosu[15], as well as Sieber[16]. Childe[17], Vogt and McKhann[5], Park[18], Mitchell[6], and Donnally, Schutz, and Nimetz[19] demonstrated that radiographs of growing bones are of diagnostic value in lead poisoning. Childe[17] observed that these white lines at the costochondral junctions are of no significance after ten years of age. Van der Plaats-Keyzer[20] believes that the increased absorption of Roentgen rays at these points can be attributed to deposition of calcium, rather than the concentration of lead. The width of these epiphyseal lines depends upon the amount of growth which has taken place during the period of lead ingestion and cannot be of prognostic importance, since only a very narrow white line may be produced by large quantities of lead ingested over a short period of time although the patient may succumb from plumbism, while small amounts of lead taken over a long period of time produce broad lines and are accompanied by mild symptoms of lead toxicosis in the patients. These lines are in no way pathognomonic of lead poisoning in either the child or adult since it has been shown that such radiographs are produced in strontium, phosphorus

and bismuth poisoning, healed and healing rickets and scurvy, marble bones, and in cases where the bone growth has ceased, such as in adults and cretins. Consequently, an indication of plumbism in children may be obtained by roentgen ray findings but a differentiation from other diseases must rest upon some pathognomonic determination.

The daily excretion of small variable amounts of lead in the urine of the vast majority of people from all parts of the universe has been reported, although Boyd and Ganguly[22] could not detect lead in the urine of Indians but demonstrated significant quantities of it in the urine of Europeans from the same locality. The excretion of lead in the urine of normal individuals was reported by Kehoe, Thamann, and Cholak[27] to vary between 0.02 to 0.08 mgm. per liter or 0.05 to 0.10 mgm. per 24 hour specimen[28]. This range of values was confirmed by Ross and Lucas[29], although Behrens and Taeger[30] reported a somewhat lower normal range. One of the factors influencing this variance has been shown by Millet[23], Newman[24], Kehoe, Thamann, and Cholak[25], among others, to be the chemical and physical characteristics of the lead salts and preparations ingested or absorbed[26]. Various amounts of lead have been reported excreted in the urine of patients showing definite symptoms of plumbism. Behrens and Taeger[30] found 0.033 to 0.394 mgm. Pb per liter, Ross and Lucas[29] reported 0.15 to 0.64 mgm. Pb per liter in battery workers, Kehoe, Thamann, and Cholak[25] observed as high as 0.52 mgm. Pb per liter, and Mitchell[6] found lead in the urine of all his cases of plumbism. Kehoe[32] states that the urine of severely exposed persons, immediately after the cessation of exposure rarely contains lead in excess of 0.30 mgm. per liter and the rate of urinary excretion drops off after cessation of exposure to lead so that frequently by the time the urine has been collected the lead content is little more than 0.15 mgm. per liter. However, it does not follow that all individuals exposed to lead will have an increased lead excretion in the urine over the normal limits. Kehoe, Thamann, and Cholak[25] found that in 356 workers exposed to the lead hazard in ethyl gasoline 25 per cent exhibited from 0 to 0.03 mgm. Pb per liter of urine

and 38 per cent from 0.04 to 0.07 mgm., which made a total of 63 per cent falling within the normal limits established by them (0.02 to 0.08 mgm. Pb per liter). Fretwurst and Hertz[31] found that lead workers with no clinical symptoms of plumbism excreted from 0.03 to 0.06 mgm. Pb per liter of urine but during lead toxemia 0.07 to 0.20 mgm. Pb per liter of urine were excreted. Litzner and Weyrauch[33] reported that the urinary excretion of lead showed no constant relationship to clinical symptoms and was of little diagnostic value, while Shiels[34] found that there appeared to be no definite relationship between the urinary lead concentration and the period of exposure to lead, and only when a lead concentration of 0.20 mgm. per liter or over occurred could the finding be taken as evidence that the subject is suffering from lead poisoning. Grignaschi[38], Labat[39], and Tompsett and Anderson[104] came to the same conclusion. It is evident from the above discussion that during periods of acute exacerbation the lead findings based on the urinary excretion values are of confirmatory rather than diagnostic import for in the majority of cases of latent and incipient plumbism the lead excreted in the urine per liter, or per 24 hour specimen, falls well within normal limits. Thus, a differential diagnosis of a possible case of lead poisoning before the onset of most of the clinical symptoms recorded under Group III, table 1, cannot be made from the urinary findings, and it would be difficult to base the prognosis of mild or inactive chronic plumbism on the urinary lead output, especially since these values will be influenced by such factors as may influence the character and amount of urine excreted among which are diseases of the kidneys, dehydration, imbibition, and edema.

The rôle that the feces play in the elimination of lead is of little diagnostic value since Aub[36], Kehoe, Thamann, and Cholak[28], Tompsett and Anderson[104], as well as many other investigators have shown that it serves as a medium for the elimination of unabsorbed ingested lead from the alimentary tract, which Vigliani and Debernardi[35] found to amount to 84 per cent of the lead taken orally.

According to Ottenberg[40], anemias may be divided into three main groups: deficiency anemias, anemias due to injury to the

blood making organs, and anemias due to the disintegration of the blood. Lead anemia belongs to both the second and third groupings, since Aub showed that it was due to abnormal destruction of red blood cells in the circulating blood and not to a diminished production of blood, except in the last stages of plumbism when a degeneration of the bone marrow may occur. Lead anemia was first recognized by Laennec, and recently Lowy and Levinson[4] considered a sudden reduction in hemoglobin associated with a proportionate fall in the red corpuscles a danger signal in lead poisoning, a view supported by the experimental work of Arthus, Lourau, and Silvestre de Sacy[41]. Although Brookfield[42] found that the immediate action of colloidal lead on the red blood cell was substantial reduction in the red blood cell count (approximately 1,000,000 cells) due to blood destruction; he also observed that patients may undergo a whole course of lead therapy without showing any great fall in the red count, any increased activity of the bone marrow, or any considerable degree of stippling of the red cells.

It has been stated by Key[43] that under conditions in which toxic agents exert an action on the bone marrow and under other conditions in which physiological demands are made, increased numbers of erythrocytes enter the blood stream. The chief characteristic of these liberated immature cells is the presence of basophilic substance. Such conditions are produced by many substances, among which may be mentioned lead, arsenic, benzol, and the effects of high altitudes.

Due mainly to the investigations of Aub and his associates[44] we know that small amounts of lead greatly alter the surface of the red blood corpuscle, causing them to shrink, rendering them incapable of swelling as do normal cells and increasing their fragility, thus causing them to hemolyze on slight trauma. Pearse[45] showed that lead increases the resistance of the mature red blood cell, but decreases the resistance of the immature corpuscles thus making these cells very fragile, while it hemolyzes the mature more readily than the immature erythrocyte. These cells lose their normal stickiness and are no longer agglutinated by sera of the different iso-agglutinating groups, a fact which may

account for some of the unexplainable reactions recorded on transfusing patients suffering from acute or chronic plumbism, since the cross-typing may be erroneous and the wrong donor selected. We have, at times, encountered severe reactions on transfusing patients who had been exposed to lead and the severity of the reaction was not diminished on subsequent transfusions. All of these changes are evidence of surface alterations in the cell. The interior of the cell is not affected, as the physiological properties of the hemoglobin remain normal. The cells become so brittle that the trauma encountered in circulating causes a marked reduction of their number in the peripheral blood. This loss in circulating red cells is compensated by a regeneration of the erythrocytes, but many of these young cells are affected by lead as soon as they enter the circulation so that their basophilic substance is coagulated, and thus they become known as stippled cells. They should be differentiated from what is commonly known as basophilic degeneration, which most authorities regard as an indication of cell regeneration, and are characterized by large clumps, rather than feathery stippling.

It has been shown by many investigators that stippled cells are not pathognomonic of plumbism, but are present and increased in many other diseases, such as: Benzene poisoning[40,47,49], arsenic poisoning[40,47], aniline and aniline products poisoning[4,48], gold injections[46], copper poisoning[49], xylene and toluene poisoning[40], poisoning by chlorinated hydrocarbons[40], pernicious anemia[42], [47,48], secondary anemias[48], hemolytic jaundice[47], polycythemia[47], leukemia[42,47,48], malignant toxemia[47], cachexia following malaria and neoplasms[42,48], change to high altitude[40].

In fact, according to Price-Jones [37], stippling occurs whenever the products of the red blood cell destruction are retained in the body.

Consequently, stippling cannot be said to be pathognomonic of lead poisoning, although it is usually present during acute stages of this disease. Johnson[7] states that clinical evidence of lead intoxication occurs when the reticulocytic count is above 4 per cent, the stippled cell count above 0.2 per cent, or both above these values. However, many of the cases of lead ex-

posure examined by him presented much higher counts in the absence of clinical symptoms, and some workers under continual exposure to lead failed to exhibit clinical evidence of lead intoxication or marked alterations in either stippled-cell or reticulocytic counts. Jones[50] described severe cases of plumbism of long duration with marked secondary anemia which showed very few basophilic cells until the patients were taken away from the source of exposure and deleading begun. Only during periods of acute exacerbation may stippling be found in large numbers in all cases, and often heavy stippling was accompanied by a feeling of well being. These observations were confirmed by Badham[51], Lehmann[52], Willcocks[53], Kehoe, Thamann and Cholak[25], and Gaul and Staud[48], among many others. This has been our experience both clinically and in experimental animals. From a perusal of table 2 it becomes evident that stippling did not precede the signs and symptoms of lead toxemia and at times it was conspicuous by its absence during the toxic episode. It often appeared after the disappearance of clinical symptoms.

It was believed by Fleckel, Chernov and Turgel[54] that the earliest and most consistent changes in the blood following exposure to lead were reticulocytosis and polychromasia which preceded the appearance of basophilic granular cells. In those diseases in which there exists an anemia the reticulocytic count will be high and in direct proportion to the severity of the anemia, provided that the bone marrow is functionally capable of response and that sufficient hematogenic material is available as shown by the investigations of Orten[55], Osgood and Wilhelm[56], Krumbhaar[57], and Sabin[58]. Jones[1] has shown that the early effect of lead on the bone marrow in many patients is of such a nature that instead of a decrease in the total number of erythrocytes there is actually a condition of polycythemia and states that an early reticulocytic response occurs prior to the development of a definite state of plumbism. Whitby and Britton[59] showed that a close relationship existed between the percentage of reticulocytes and the sum of the percentage of stippled and polychromatic cells, but the stippled cells appeared later than the polychromatic cells in induced plumbism.

McCord, Holden, and Johnston[63,64] believe that polychromasia (polychromatophilia), punctate stippling and reticulation are but different manifestations of one phenomenon—the presence of basophilic substance. Its probable form in the unaltered blood is the picture observed as polychromasia. Depending

TABLE 2

Relation of basophilic stippling, reticulocytic count, and basophilic aggregation to the development of clinical symptoms in acute, chronic, and induced plumbism

	STIPPLING				RETICULOCYTES			BASOPHILIC AGGREGATES			
	None	Few-0.2%	0.2-0.9%	Over 1%	None-1.5%	1.0-4.0%	Over 4%	None-1%	1.0-1.5%	1.5-2.0%	Over 2%
Total number of cases examined		101				45			58		
During symptoms of group I	14	7	0	1	0	0	0	2	1	0	4
Preceding onset of symptoms of group II and III:											
Over 28 days	0	0	0	0	0	0	0	0	0	0	0
28-14 days	0	1	0	0	0	0	0	2	0	0	0
14-7 days	0	1	0	0	0	0	0	0	1	0	0
7-0 days	0	3	0	0	3	0	0	0	1	1	1
Total	0	5	0	0	3	0	0	2	2	1	1
During toxicosis symptoms of group II and III	27	11	3	1	17	8	2	9	2	6	5
Following subsidence of symptoms:											
0-7 days		4	1	2	3	0	2	4	1	0	4
7-14 days		3	3	3	2	2	0	1	2	0	0
14-28 days		5	1	1	2	1	0	2	0	1	2
Over 28 days	3	2	1	3	3	0	0	4	0	1	1
Total	3	14	6	9	10	3	2	11	3	2	7

upon the staining method employed, Jones[1,50] demonstrated that the basophilic substance can be made to take the form of polychromatophilia, punctate basophilia, or reticular designs. Pappenheim[60], Schilling-Torgau[61], Key[43], Brookfield[42], Whitby and Britton[59], Hawes[62], and many other investigators believe that

polychromasia, stippling, and reticulation are all due to the presence of basic staining cytoplasm of youthful origin, and are all substantially the same substance. Since these basophilic formations do not exist as such in the blood stream, McCord and associates devised a test based upon the enumeration of the total number of basophilic containing cells in the blood stream (basophilic aggregation), in distinction to stippled cells. They found that the blood of the normal adult rarely contained more than 1 per cent of basophilic erythrocytes, while workers absorbing lead without clinical manifestation and in early lead poisoning showed values ranging from 1.5 to 4.0 per cent and as high as 20 per cent. In the absence of any other pathology, values in lead exposed workers in excess of 1.5 and particularly 2.0 per cent are suggestive of lead absorption and the possibility of approaching clinical lead poisoning. One to 1.5 per cent represents the threshold zone in which interpretations are open to doubt. It would appear that pathological conditions other than lead poisoning in which stippling was produced would give positive findings by this test and such was the case as Gould, Kullman and Shecket[65] have recently shown. They found that basophilic aggregation percentages were unreliable. In only 25 per cent of their counts was it higher than the stippled cells percentage, and, on every occasion, the reticulocytic count was higher than the basophilic aggregate. We have noted that in anemia, caused by loss of blood following a radical breast amputation, the basophilic aggregate percentage increased from 0.46 to 1.80 per cent. The subsequent introduction of 268 mgm. of lead (as colloidal lead-manganese phosphates) resulted in a drop to 0.98 per cent. In the case of an exploratory thoracotomy the basophilic aggregate was 1.1 per cent and after the intravenous injection of 344 mgm. lead (as colloidal lead-manganese phosphates) it dropped to 0.76 per cent; a result which makes this test of little prognostic value in following surgical cases complicated by mild lead toxemia or vice versa. From the results recorded in table 2 it becomes evident that stippling did not occur in 38 out of 101 cases of plumbism and 35 additional cases showed stippling within normal limits. Basophilic aggregation

was present in all the cases examined but 25 of these cases fell within the range of normal limits and an additional 8 cases were in the so called threshold zone. No reticulocytes were found in 7 out of 45 cases and 30 of these cases were within normal limits. These results indicate that although a reticulocytic response occurs prior to the development of a definite state of lead toxicosis, it makes its appearance at approximately the same time as the clinical symptoms of Group III, table 1, and generally lags behind them but precedes stippling. From these results (table 2) we are inclined to agree with Krafka[66] as well as Haemisch[107] that the quantitative estimation of reticulocytes, punctiphilia, polychromatophilia and normoblasts, to which should be added basophilic aggregation, has little value in establishing a prognosis in lead poisoning, since at the very time the lead is acting, the count may be very low and no correlation exists between fatalities and blood crises. The presence of many pathological conditions often makes these findings of little diagnostic value and of no differential importance.

Although it was shown that the effect of lead is to destroy the red blood cell by surface action, according to Aub, Fairhall, Minot, and Reznikoff[44], lead exhibits some inhibiting effect on the phagocytizing ability of leucocytes, does not affect their surface to any observable degree, but exerts a poisoning action due to its absorption within the cell; an observation which supports the findings of Fine[67] and Fenn[68]. Petrov[69] has shown that there is a rise in the lymphocytes, monocytes, and a general shift to the left of the Schilling Index in the early stages of plumbism. Some cases were characterized by a leucocytosis, others by a leucopenia. Doan and Wiseman[70] have shown that under pathologic conditions a shift to the left complicates the cell picture by the introduction of monoblasts, myeloblasts and lymphoblasts. According to Carrel[71] the aspect of monocytes cultivated in a given serum expresses simultaneously the individual characteristics of this serum and the modifications of those characteristics under the influence of pathological agencies. Brookfield[42] reported an increase in monocytes (from 2 to 5 per cent) during induced lead toxicosis which he believed due to a

stimulation of the reticulo-endothelium by lead. Lenzi[72] looked upon lead monocytosis as a manifestation common to all forms of poisoning from heavy cations. Ferguson and Ferguson[73] believed that the ratio of the large to small lymphocytes was of import in the early diagnosis of plumbism. Seitz[74] called attention to this lymphocytosis in lead poisoning which was confirmed by the investigations of Lenzi[72], but the latter claimed that it was not specific for plumbism as it occurred in many types of intoxication. Selig[75], as well as Biondi[76] reported an eosinophilia during lead toxemia. The former found as much as 70 per cent, but the latter considered it as having only slight relative importance in plumbism. Gould, Kullman, and Shecket[65] found only normal eosinophilic counts throughout the entire course of lead intoxication. These same investigators reported that basophilic (toxic) granulation was a constant finding in the cytoplasm of the neutrophils in all the cases which came under their study. Kasahara and Nagahama[77] reported more pathologic leucocytic granules in lead meningismus than in lead anemia, and, although they usually paralleled the basophilic granulation of erythrocytes, they did not decrease in cases of lead meningismus with bad prognosis. Bethell[78] showed that basophilic granulation of the neutrophiles was not limited to infectious processes, but may occur in such conditions as reactions following transfusion with incompatible blood, intensive roentgen therapy, toxemias of pregnancy, extensive malignancy with necrosis, and chronic myelogenous leukemia. The granules are predominant in cells most recently released into the circulation. A leukocytosis, usually marked, was noted in most of Gould, Kullman and Shecket's[65] cases in about three and a half weeks after the beginning of treatment. Myelocytes were found in 9 out of 22 cases in the peripheral blood two weeks or more after the beginning of lead therapy, although Schmahl[79] showed that a marked reduction in all forms of the myelocyte was caused by lead in myelogenous leukemia. Nestsadimenko and Volkow[80] confirmed Petrov's finding of a left shift in the neutrophiles in acute lead poisoning, but in chronic lead poisoning Ferguson and Ferguson[73] showed that this left shift of the

TABLE 3

Leukocytic shifts during the various stages of plumbism

PATIENT DESIGNATION	CLINICAL SYMPTOMS ACCORDING TO TABLE 1	HEMOGLOBIN	TOTAL RED BLOOD CELLS	TOTAL WHITE BLOOD CELLS	LETHAL INDEX	MULTIPLE INDEX	SCHILLING INDEX	NEUTROPHILES						LYMPHOCYTES					MONOCYTES	EOSINOPHILES	BASOPHILES	TOXIC GRAN.	NEUTROPHILES	SEGMENTERS	TYPE OF SHIFT	BLOOD PLATELETS	ETIOLOGY	ASSOCIATED DISEASES
								Myeloblast	Myelocyte	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblast	Large	Medium	Small	Total lymphocytes										
		grams per 100 ml.	*per million*	*per 1,000*																			*per mm.³*	*per mm.³*		*per 1,000*		
McM.	Group I	14.5	4.00	7.3	0.08	6.8	0.43	0	4	1	17	51	73	0	0	0	20	20	5	1	1	0	5,329	3,723	4		Unknown	None
	Group II	15.4	4.31	6.2	0.04	9.0	0.56	0	0	4	24	50	78	0	0	0	18	18	0	2	2	0	4,836	3,100	4			
W. N.	None	8.5	3.63	9.8	0.03	32.0	2.00	0	0	2	53	28	83	0	0	7	0	7	8	0	2	10	8,134	2,744	2	289	Induced	Malignancy
	Group II	10.5	4.01	10.5	0.01	11.0	0.70	0	0	1	29	43	73	0	7	6	1	14	13	0	0	1	7,665	4,515	4	242		
A. S.	Group I	9.8	3.43	116.2	1.00	66.5	4.16	7	11	9	48	18	93	0	1	1	0	2	2	0	3	0	108,066	20,916	2	474	Induced	Leukemia
	Group I	10.5	3.36	65.8	2.00	87.3	5.46	11	19	18	34	15	97	0	0	0	0	0	2	1	0	0	63,826	9,870	2	426		
	Group II	10.0	3.04	57.6	3.00	123.2	7.70	16	16	20	33	11	96	0	0	0	1	1	1	0	2	0	55,296	6,336	2	354		
	None	11.5	3.93	54.9	1.00	41.6	2.60	3	25	11	29	26	94	0	0	1	0	1	2	3	0	0	51,606	14,274	2	384		
	None	10.1	3.41	119.8	2.80	137.0	8.60	8	20	18	40	10	96	0	0	0	1	1	2	0	1	0	115,008	11,980	2	328		
	None	10.8	3.56	104.8	10.75	360.0	22.50	13	30	17	30	4	94	0	1	1	0	2	1	3	0	0	98,512	4,192	2	446		
	Group II	10.2	3.75	13.6	0.07	10.0	0.60	0	4	3	26	52	85	0	2	3	2	7	1	4	3	0	11,560	7,072	2	114		
W. R.	None	11.5	4.16	5.8	0.08	10.8	0.68	0	0	0	26	38	64	0	3	8	3	14	13	8	1	10	3,712	2,204	4	238	Induced	Malignancy
	None	10.5	3.97	7.7	0.09	12.2	0.76	0	0	0	30	39	69	0	0	11	7	18	10	3	0	10	5,313	3,003	4	276		
	None	9.8	4.24	4.0	0.07	10.0	0.60	0	0	0	25	41	66	0	0	4	17	21	11	0	2	5	2,640	1,640	4	344		
	Group III	10.5	3.62	5.4	0.13	16.0	1.00	0	0	0	29	27	56	0	4	11	9	24	17	1	2	3	3,024	1,458	4	322		
O. H.	None	10.0	3.99	8.4	0.13	17.6	1.10	0	0	0	31	28	59	0	0	0	34	34	5	1	1	0	4,956	2,352	4	364	Induced	Malignancy
	None	10.2	3.76	7.7	0.03	4.0	0.25	0	0	0	10	40	50	0	0	13	24	37	11	0	2	0	3,850	3,080	4	290		
	None	10.5	3.64	8.7	0.03	5.0	0.30	0	0	0	15	48	63	0	1	21	10	32	5	0	0	0	5,481	4,176	4	254		
	None	10.5	3.74	6.1	0.03	4.0	0.26	0	0	0	13	50	63	0	0	19	9	28	8	0	1	0	3,843	3,050	4	220		
	Group I	10.3	3.58	5.9	0.06	8.0	0.50	0	0	0	20	40	60	0	0	0	32	32	7	1	0	0	3,540	2,360	4	282		
T. C.	None	14.5	4.38	7.9	0.02	13.0	0.80	0	1	1	38	48	88	0	0	3	6	9	2	1	0	0	6,952	3,792	2	426	Induced	Malignancy
	Group III	14.3	4.79	5.5	0.07	9.4	0.59	0	0	0	29	49	78	0	4	6	8	18	4	0	0	0	4,290	2,695	4	312		

	Group III	14.3	4.79	5.5	0.07	9.4	0.59	0	0	0	29	49	78	0	4	6	8	18	4	0	0	0	4,290	2,695	4	312	Induced	Malignancy
J. G.	Group I	11.8	3.81	15.8	0.07	10.0	0.60	0	0	0	26	42	68	0	2	12	8	22	8	2	0	2	10,744	6,636	4	334	Induced	Malignancy
	Group II	11.1	4.50	8.4	0.11	14.2	0.89	0	0	0	41	46	87	0	1	6	2	9	3	1	0	0	7,308	3,864	4	292		
	Group III	11.0	4.38	9.1	0.13	17.0	1.08	0	0	0	39	36	75	0	0	4	14	18	4	2	1	0	6,825	3,276	4	272		
Plumbism difficult of clinical diagnosis																												
M. J.	Group I	11.8	3.32	11.0	0.13	17.6	1.10	0	0	0	41	37	78	0	1	8	6	15	5	2	0	15	8,580	4,070	4	340	Induced	Malignancy
E. J.	Group II	13.3	4.94	11.1	0.01	10.8	0.68	0	0	1	33	50	84	0	1	9	1	11	5	0	0	0	9,324	5,550	4	354	Industrial	None
B. A.	Group II	13.8	4.42	23.1	0.51	20.8	1.30	4	14	7	22	35	82	0	0	3	3	6	6	1	5	10	18,942	8,085	2	390	Induced	Leukemia
T. C.	Group II	14.5	4.38	7.9	0.02	13.0	0.80	0	1	1	38	48	88	0	0	3	6	9	2	1	0	0	6,952	3,792	2	426	Induced	Malignancy
J. W.	Group I	13.4	4.70	15.2	0.08	11.0	0.69	0	0	0	29	42	71	0	0	9	7	16	11	0	2	5	10,792	6,384	4	240	Induced	Malignancy
W. K.	Group I	12.5	4.65	6.9	0.13	17.0	1.06	0	0	0	34	32	66	0	0	10	8	18	14	2	0	30	4,554	2,208	4	392	Unknown	None
L. M.	Group I	5.5	2.66	8.4	0.01	22.6	1.41	0	0	1	43	31	75	0	2	11	1	14	8	2	1	10	6,300	2,604	4	424	Induced	Malignancy
L. R.	Group I	12.5	4.39	7.7	0.05	6.0	0.40	0	0	0	23	54	77	0	0	1	5	6	11	6	0	0	5,929	4,158	4	302	Unknown	None
B. L.	Group II	11.1	4.11	13.0	0.02	14.0	0.88	0	1	1	30	36	68	0	0	1	27	28	2	0	2	0	8,840	4,680	4	350	Induced	Malignancy
Plumbism easy of clinical diagnosis																												
W. N.	Group II	10.5	4.01	10.5	0.01	11.0	0.69	0	0	1	29	43	73	0	7	6	1	14	13	0	0	5	7,665	4,515	4	242	Induced	Malignancy
H. H.	Group III	7.5	2.48	542.0	6.90	144.0	9.00	43	26	12	9	10	100	0	0	0	0	0	0	0	0	0	54,200	5,420	2	428	Unknown	Leukemia
T. C.	Group III	14.3	4.79	5.5	0.07	9.4	0.59	0	0	0	29	49	78	0	4	6	8	18	4	0	0	0	4,290	2,695	4	312	Induced	Malignancy
B. G.	Group II	9.0	3.82	4.5	0.08	10.7	0.67	0	0	0	27	40	67	0	6	12	3	21	9	3	0	0	3,015	1,800	4	438	Induced	Malignancy
McC.	Group II	15.4	4.31	6.2	0.04	9.0	0.56	0	0	4	24	50	78	0	0	0	18	18	0	2	2	0	4,836	3,100	4		Industrial	None
L. R.	Group II	12.5	4.18	5.6	0.03	17.7	1.11	0	1	1	28	26	56	0	1	12	9	22	18	4	0	5	3,136	1,456	4	254	Induced	Malignancy
G. H.	Group II	13.1	3.53	21.6	0.12	16.0	1.00	0	0	0	36	35	71	0	1	14	9	24	3	2	0	10	15,336	7,560	4	938	Induced	Malignancy
W. R.	Group III	12.8	4.41	4.8	0.17	21.9	1.37	0	0	0	40	29	69	0	7	5	8	20	10	1	0	8	3,312	1,392	4	414	Induced	Malignancy
E. J.	Group III	13.5	5.48	10.0	0.01	11.0	0.71	0	0	1	32	46	79	0	0	6	3	9	11	1	0	0	7,900	4,600	4	376	Industrial	None
J. G.	Group III	11.0	4.38	9.1	0.14	17.0	1.08	0	0	0	39	36	75	0	0	4	14	18	4	2	1	0	6,825	3,276	4	272	Induced	Malignancy
S. V.	Group III	12.5	5.61	7.7	0.24	32.0	2.00	0	0	0	51	26	77	0	4	5	1	10	13	0	0	0	5,929	2,002	4	274	Induced	Malignancy
W. H.	Group III	11.0	4.10	9.4	0.01	1.8	0.11	0	0	0	6	54	60	0	8	4	14	26	14	0	0	0	5,640	5,076	4	272	Industrial	None
K. L.	Group III	6.5	2.99	9.0	0.04	40.9	2.56	0	0	2	57	23	82	0	1	7	4	12	6	0	0	5	7,380	2,070	2	170	Induced	Malignancy
Approaching clinical crisis																												
W. N.	Group III	10.5	4.01	10.5	0.01	11.0	0.70	0	0	1	29	43	73	0	7	6	1	14	13	0	0	1	7,665	4,515	4	242	Induced	Malignancy
J. H.	Group III	12.5	5.11	8.0	0.05	7.0	0.46	0	0	0	23	50	73	0	2	4	4	10	16	1	0	0	5,840	4,000	4	280	Industrial	None
L.	Group II	10.5	4.11	181.8	13.00	384.0	24.00	22	18	14	19	3	76	0	0	2	1	3	0	4	17	0	138,168	5,454	2	1,292	Induced	Leukemia
T. Mc.	Group II	15.4	4.31	6.2	0.04	9.0	0.56	0	0	4	24	50	78	0	0	0	18	18	0	2	2	0	4,836	3,100	4		Unknown	None
K. L.	Group III	6.5	2.99	9.0	0.04	40.0	2.50	0	0	2	57	23	82	0	1	7	4	12	6	0	0	5	7,380	2,070	4	170	Induced	Malignancy
B. G.	Group III	9.0	3.82	4.5	0.08	10.7	0.67	0	0	0	27	40	67	0	6	12	3	21	9	3	0	0	3,015	1,800	4	438	Induced	Malignancy
L. M.	Group III	5.1	2.02	7.6	0.16	21.0	1.31	0	0	0	42	32	74	0	0	4	4	8	15	3	0	8	5,624	2,432	4	310	Induced	Malignancy
S. V.	Group III	12.5	5.61	7.7	0.25	32.0	2.00	0	0	0	51	26	77	0	4	5	1	10	13	0	0	0	5,929	2,002	4	274	Induced	Malignancy

neutrophiles changes over into a right shift. Our own experience with horses showed that the intravenous injection of lead preparations was followed by a leucopenia which changed over into a leucocytosis, accompanied by a slight rise in the monocytes which fluctuated over a period of nine hours. There was a shift to the left of the neutrophiles corresponding to the type 4 shift of Crocker and Valentine[81,82] which changed over into a type 2 shift in both human plumbism (table 3) and induced lead toxemia in the horse.

The effect of lead upon the blood platelets has not been extensively studied, but since foreign substances are often entrapped by platelet clumps before being phagocytized by the leucocytes, as demonstrated by finding such conglomerates within the body of the phagocytizing cells, we would be drawn to believe that lead would have more than a negligible effect upon the platelets and indirectly influence phagocytosis. In our own experience it has been observed that the intravenous injection of colloidal lead preparations reduced thrombocytes.

In the analysis of the shiftograms and hemograms of table 3 it is of importance to appreciate that the change in the leucocytes apparently precedes the development of the recognized clinical symptoms of plumbism. Thus a change may take place in the type of shift, while the symptomatology would seem to indicate a different effect due to the frequent lag of clinical signs behind the leucocytic change. Particularly is this true since the hemograms comprising the shiftograms were taken at 48 hour intervals. A study of the hemograms in table 3 indicates that in every case of mild lead toxemia a type 4 shift and hemogram of Crocker[81] was found, which, as the toxic condition increases to the acute stage, gradually change over into a type 2 shift and hemogram. The hemographic alterations in patients who contracted plumbism from the therapeutic intravenous injections of lead (as colloidal preparations), in known industrial lead exposure, and in symptomatic cases of unknown etiology have been studied in table 3. We have come to the conclusion by the use of the entire hemogram, as well as the shiftogram, in the manner so ably demonstrated by Crocker and Valentine[81,82], that the entire leucocytic picture, including the determination of toxic granules, provides

a method for the identification of the early toxicosis which precedes the signs and symptoms of plumbism. However, this hemographic indication is not pathognomonic of lead poisoning. It is true for any toxicosis of this general nature. A type 4 shift and shiftogram has been shown by many investigators[83] to be characteristic of most toxic conditions in the body caused by infections, subacute stimulations, many of the poisonous intoxications and the lymphomonocytic phase of healing convalescence. Consequently, one must resort to a differential diagnostic procedure. This can be accomplished by an accurate determination of lead in the serum, cells and fibrin fraction of the blood when taken into consideration with the total amount of lead in the whole blood. It is possible, by this procedure, to make a differentiation between acute, chronic, and mild lead poisoning, as well as to differentiate between mild lead toxemia and such conditions as gastro-enteritis, hypercalcemia, food poisoning, sun and x-ray burns, or colics from other causes. The therapeutic use of lead can be controlled by this procedure since treatment may be instigated before an acute lead crisis develops.

ANALYTICAL PROCEDURE

Ten grams of venous blood (approximately 10 ml.) is procured by means of a glass syringe and needle which has been treated for the removal of lead with HNO_3, double distilled water, and a KCN-diphenylthiocarbazone solution.[84] After the removal of the needle from the syringe, the blood is transferred to a tared, 25 ml., specially cleaned, glass-stoppered Pyrex flask. It is essential that all glassware be free from lead, since amounts of this metal which are left in the flasks, syringes and needles after the ordinary laboratory cleaning procedure, even when followed by a distilled water rinse, is sufficient to cause a definite error in the results.[84, 85, 106] The blood is then defibrinated by a rotary shaking motion and weighed. The flask is then refrigerated until the serum starts to separate. At this point the blood is transferred to a graduated Pyrex centrifuge tube and capped with filter paper, the fibrin ball remaining in the flask. The tube is recooled and centrifuged at a speed of 2100 r.p.m. (radius of centrifuge arm 12 cm.) for twenty minutes. At the completion of centrifugalization the tube should be cool to the touch. The total volume of fluid in the tube is then decanted off into a tared 100 ml. Kjeldahl flask and weighed. The volume of the remaining fluid is noted and the contents transferred to a 300 ml. Kjeldahl flask to which the fibrin is added. The samples in both flasks are then independently digested by the wet process and the lead determined by the diphenylthiocarbazone method of Wilkins, Willoughby, Kraemer and

Smith[85] with this difference: the lead is extracted with one 20 ml. portion of 1 per cent nitric acid instead of two 10 ml. portions.

Defibrinated blood and serum were used in preference to the employment of an anticoagulant so as to minimize any changes in equilibrium of serum and cell lead, since it was demonstrated by Ponder,[86] Vaughan and Goddard,[39] and others, that this procedure eliminates the need for the introduction of leakage factors. Ponder and Robinson[87] also showed that there is a loss of osmotically active substances into the surrounding hypotonic medium, whenever the medium in which the cell is suspended differs more than slightly from the normal environment. These investigators, as well as Magath and Hurn[88] found that the tonicity, shrinkage and the fraction of total water which is apparently free in the cells are decreased and the fragility increased in oxalated blood. Yoshimura[89] reported a shift to the acid side when clotting has been prevented by means of any anticoagulant with the exception of 1:10 parts of a 1 per cent heparin solution. Mermod and Dock[90] demonstrated the disintegration of reticulocytes by isotonic citrate or oxalate solutions and Schmidt[91] showed the reduction of phospholipide content of oxalated plasma is due to alterations produced by the oxalate in cell and plasma volumes and not to precipitation. Sperry and Schoenheimer[92] found that oxalated plasma contains significantly smaller amounts of total and free cholesterol than either the serum or heparinized plasma from the same blood.

The most satisfactory method for defibrinating blood was found to be a judicious freezing out of the serum with carbon dioxide snow, but this method was discarded, as well as that of Bellis and Scott[93] because of the difficulties encountered by its use in the clinic and wards.

The lead content of the whole blood, serum, and cells and fibrin fraction is then calculated and reported on an arbitrary 10 gram basis, so that blood from different individuals, as well as results from a series of samples taken from the same patient over a considerable period of time may be compared without having to take into consideration the hematocrit reading, the size of the corpuscles, the packing effect and particularly the changes in the consistency and volume of the blood which result from such pathological conditions as edema, dehydration and imbibition. Otherwise, if the findings were reported on 10 grams of blood, all of these factors would have to be known in order to compare values from different individuals and from the same individual when the samples were taken at different times under different pathological conditions, for example anemia, starvation and vitamin deficiencies.

BLOOD LEAD CONTENT AS AN INDICATION OF ACUTE, CHRONIC, AND LATENT PLUMBISM

Lead is a common finding in the peripheral blood of almost all individuals and during recent years extensive investigations

have been made to show a relationship between the chemical evaluation of blood lead and lead poisoning. Tompsett and Anderson[104] observed that the lead content of the blood gives a better picture of plumbism than that of the urine or feces, since in the case of the urine the concentration of lead varies with the volume secreted. Litzner and Weyrauch[33,94a], and Bass[94], Schmitt and Taeger[95], Blumberg and Scott[96], and McMillen and Scott[97] have shown that the lead content of blood during plumbism bears a close relationship to the appearance of clinical symptoms, but the difference between health and lead poisoning is purely quantitative rather than qualitative. These latter investigators state that the detection of pathological blood lead has been particularly valuable in differentiating lead encephalopathy from other possible causes of convulsions in patients[98], and they find that clinical lead poisoning has never been observed with a blood lead value below 0.01 mgm. Pb per 10 ml., while in severe lead poisoning values above 0.02 mgm. per 10 ml. were obtained. Kehoe, Thamann and Cholak[28] place the normal blood level of American medical students at 0.006 mgm. per 10 ml. of blood. Our associates, Willoughby and Wilkins,[106] have found a slightly lower value (0.0025 $\pm$0.0002 mgm. per 10 grams) in a series of healthy individuals and hospitalized patients without symptoms of plumbism.

The greater part of the blood lead in experimental acute lead poisoning was shown by Aub, Fairhall, Minot, and Reznikoff[44], as well as Teisinger[99] to be held by the plasma and but little by the cells. Blumberg and Scott[100] by the analyses of control bloods, including a hemophilic sample, found that the cell fraction contained at least half and usually much more, of the non-pathological blood lead, but due to the possible changes in the distribution ratio they preferred to base their diagnosis of lead poisoning on the whole blood values. Our findings support the contention that the pathological lead in plumbism is the serum or circulating lead and that this differs from the non-pathological, or inactive lead stored in the tissues, cells and organs. Teisinger[99] showed that the circulating lead was present

as an organic complex and not as the phosphate. Jowett[101] believes that this lead complex is composed of an organic phosphate, calcium and chlorine, while Aub[36] states that in chemical systems as complicated as blood plasma, an equilibrium of several such compounds is not unreasonable and any of these compounds might remain ionized and dispersed in the presence of plasma proteins. This indicates that serum lead should hold a definite relationship to clinical symptoms in lead poisoning and this was shown to be true by Wexler and Sobel[102].

Since lead is a constant finding in the blood of most persons, a so called normal range of serum and whole blood lead values is established in table 4 in order to compare the blood lead of healthy individuals with that of patients showing clinical symptoms of lead poisoning. Table 5 shows that there is no essential difference in the lead content of the blood fractions in health and disease providing lead toxicosis is not present. The tables show that the range of lead values is the same for both male and female. Climatic, seasonal, and daily changes had no effect. From table 6 it may be seen that this range of values is not affected by the time of day the blood samples were taken, daily fatigue, nor the quantity of food eaten or digested. Table 7 shows that violent exercise, such as 10 minutes of continuous stair running, has no influence on these values.

Menstruation had no effect upon the limits of the normal (healthy) lead range (table 8), as essentially the same values were obtained during the first, second, and third days of menstruation, the end of the period and about the middle of the quiescent stage. In one individual, ovulation, as determined by the method of Latz and Reiner[103], showed no effect.

Thus the range for lead in the blood of healthy individuals, as well as patients hospitalized for other causes than lead poisoning and free from symptoms of this disease, was found to lie between the following limits:

MGM. OF LEAD PER 10 GRAMS	SERUM	CELL AND FIBRIN FRACTION	WHOLE BLOOD
Minimum	0.000	0.002	0.001
Maximum	0.000	0.011	0.006

TABLE 4

Lead content of the blood of healthy normal individuals

DESIGNATION	SEX	OCCUPATION	LEAD IN MGM. PER			DATE
			10 grams serum	10 grams cells and fibrin	10 grams whole blood	
M. S.	F	Nurse	Nil	0.001	0.001	July 19
F. K.	F	Technician	Nil	0.002	0.001	December 12
M. E. N.	F	Nurse	Nil	0.002	0.001	January 10
M. P.	F	Nurse	Nil	0.003	0.002	December 9
E. P.	F	Nurse	Nil	0.003	0.002	January 9
E. B. R.	F	Nurse	Nil	0.004	0.002	January 10
C. L. S.	F	None	Nil	0.004	0.002	November 29
M. M.	F	Nurse	Nil	0.006	0.003	January 10
D.	F	Nurse	Nil	0.006	0.003	January 9
E. Y.	F	Nurse	Nil	0.006	0.003	January 9
J. G.	F	Artist	Nil	0.007	0.003	March 10
M. B.	F	Nurse	Nil	0.007	0.003	January 10
G. D.	F	None	Nil	0.007	0.003	September 24
F. G.	F	Nurse	Nil	0.007	0.003	January 9
L. Y.	F	Nurse	Nil	0.007	0.004	January 9
M. V.	F	Technician	Nil	0.007	0.004	April 1
F. V.	F	Secretary	Nil	0.007	0.004	November 12
M. C.	F	Nurse	Nil	0.007	0.004	December 9
L. D. G.	F	Secretary	Nil	0.007	0.004	November 12
S. B. B.	F	Secretary	Nil	0.007	0.004	January 9
M. M.	F	Nurse	Nil	0.007	0.004	December 12
L. K. P.	F	Technician	Nil	0.008	0.004	November 19
M. C.	F	Technician	Nil	0.008	0.004	December 9
V. L. B.	F	Secretary	Nil	0.011	0.005	November 12
G. E. M.	M	Doctor	Nil	0.004	0.003	January 9
T. K. R.	M	Doctor	Nil	0.006	0.003	January 9
W. A. K.	M	Doctor	Nil	0.006	0.003	November 10
R. M. S.	M	Doctor	Nil	0.006	0.003	January 21
F. L. S.	M	Doctor	Nil	0.007	0.004	November 12
G. M.	M	Janitor	Nil	0.007	0.004	November 19
L. P. H.	M	Doctor	Nil	0.007	0.004	January 10
H. L. S.	M	Doctor	Nil	0.007	0.004	January 10
C. N. D.	M	Doctor	Nil	0.007	0.004	July 19
T. L. W.	M	Doctor	Nil	0.008	0.004	November 19
F. L. M.*	M	Doctor	Nil	0.008	0.005	November 13
J. O. G.	M	Doctor	Nil	0.008	0.005	May 27
Normal range:						
Minimum			Nil	0.001	0.001	
Maximum			Nil	0.011	0.005	

* Hemophiliac.

Nil: no lead definitely detected beyond the limits of experimental error of the analytical method used.

TABLE 5

Distribution of lead in the blood associated with pathological processes other than plumbism

CASE NUMBER	SEX	AGE	DIAGNOSIS	LEAD IN MGM. PER			DATE
				10 grams serum	10 grams cells and fibrin	10 grams whole blood	
1451	F	48	Sq. cell carc. esophagus	Nil	0.004	0.001	January 4
P. G. H.	F	39	Ulcer of the stomach	Nil	0.003	0.001	July 20
1130	F	56	Carcinoma of breast	Nil	0.003	0.002	August 24
F.	F	43	Chorio-epith.	Nil	0.003	0.002	May 24
G.	F	10	Chronic nephritis	Nil	0.003	0.002	July 19
575	F	34	Carcinoma of ovary	Nil	0.005	0.002	January 14
1022	F	62	Myelogenous leukemia	Nil	0.004	0.002	February 15
1883	F	78	Carc. gall bladder with met.	Nil	0.004	0.002	November 27
S. B.	F	38	Chronic inflammation	Nil	0.004	0.002	October 31
725	F	21	Myelogenous leukemia	Nil	0.005	0.002	October 20
700	F	8	Erytho-blastic anemia	Nil	0.006	0.002	June 25
1257	F	22	Monocytic leukemia	Nil	0.008	0.002	November 5
P. G. H.	F	35	Brain abscess	Nil	0.004	0.003	May 27
949	F	23	Adenocarcinoma of rectum	Nil	0.005	0.003	April 20
1493	F	45	Carcinoma of breast	Nil	0.006	0.003	February 11
924	F	51	Scirrhous carc. mammary gland	Nil	0.007	0.003	April 23
1758	F	32	Rec. carc. rt. breast w. met.	Nil	0.007	0.003	November 1
1190	F	62	Met. carc. rt. side of neck	Nil	0.007	0.003	November 23
M.	F	50	Glioma	Nil	0.007	0.004	May 27
1862	F	34	Met. carc. lt. breast	Nil	0.007	0.004	January 22
A. B.	F	39	Tumor lt. lobe cerebellum	Nil	0.007	0.004	January 23
G. F.	F	56	Carc. of breasts with met.	Nil	0.007	0.004	February 16
952	F	61	Carcinoma of lt. breast	Nil	0.008	0.004	May 21
933	F	53	Scirrhous carc. rt. breast	Nil	0.009	0.004	May 28
897	F	73	Rec. carc. rt. breast	Nil	0.009	0.005	March 27
C. M.	F	53	Carcinoma of lt. breast	Nil	0.009	0.005	February 14
M. H.	F	46	Sarcoma rt. breast	Nil	0.010	0.005	June 24
1562	F	57	Adenocarcinoma of uterus	Nil	0.012	0.005	April 16
2120	F	61	Sq. cell carc. cervix	Nil	0.011	0.006	December 28
1038	F	33	Melanotic sarc. rt. eye	Nil	0.013	0.006	August 18
1403	M	14	Myelogenous leukemia	Nil	0.002	0.001	October 23
G. S.	M	22	Fracture	Nil	0.003	0.002	December 13

Nil: no lead definitely detected beyond the limits of experimental error of the analytical method used.

TABLE 5—*Concluded*

CASE NUMBER	SEX	AGE	DIAGNOSIS	LEAD IN MGM. PER			DATE
				10 grams serum	10 grams cells and fibrin	10 grams whole blood	
1352	M	50	Malignant tumor of lymph-node	Nil	0.004	0.002	December 3
1448	M	49	Adenocarcinoma of thyroid	Nil	0.004	0.002	January 8
1301	M	31	Teratoma of testicle	Nil	0.005	0.003	September 12
1171	M	51	Epithelioma lower lip	Nil	0.005	0.003	April 22
1564	M	39	Carcinoma of stomach	Nil	0.007	0.003	April 22
935	M	29	Hydrogen sulphide poisoning	Nil	0.007	0.004	April 28
1852	M	26	Sarcoma lt. groin	Nil	0.007	0.004	January 20
909	M	58	Sq. cell carc. esophagus	Nil	0.006	0.004	April 2
1730	M	24	Hodgkin's disease	Nil	0.008	0.004	November 11
931	M	27	Tumor rt. maxillary antrum	Nil	0.009	0.004	April 26
932	M	65	Sq. cell carc. lower lip	Nil	0.011	0.004	April 26
719	M	62	Carcinoma of bladder	Nil	0.009	0.005	September 27
1786	M	29	Hodgkin's disease	Nil	0.010	0.005	November 12
2112	M	48	Carcinoma of chest wall	Nil	0.010	0.006	December 21
944	M	41	Hodgkin's disease	Nil	0.011	0.006	June 7
Normal range:							
Minimum				Nil	0.002	0.001	
Maximum				Nil	0.013	0.006	

It is important to note that in no instance was lead detected in the serum fraction of the blood in this group of individuals by the analytical method employed. This has been sustantiated by Willoughby and Wilkins[106] in a recent series of patients without signs or symptoms of plumbism.

In evaluating these tables it is of importance to appreciate that often it was necessary to determine quantities of lead in the neighborhood of the error of the analytical method employed[85]. Willoughby and Wilkins[106], in a recent publication, showed that this error lies between 0.0005 and 0.0010 mgm. in approximately 98 per cent of their blood samples to which a known amount of lead was added (0.002 to 0.010 mgm. lead per 10 grams blood), obviously, our results may be in error by this amount. Con-

TABLE 6

Lead content of the blood of healthy individuals: effects of daily fatigue and nourishment

DESIGNATION	SEX	TIME OF DAY	NOURISHMENT	LEAD IN MGM. PER		
				10 grams serum	10 grams cells and fibrin	10 grams whole blood
F. L. S.	M	9:30 a.m.	Before	Nil	0.007	0.004
		2:30 p.m.	After	Nil	0.007	0.004
		5:30 p.m.	Before	Nil	0.007	0.004
T. L. W.	M	9:30 a.m.	Before	Nil	0.008	0.004
		2:00 p.m.	After	Nil	0.008	0.004
		5:00 p.m.	Before	Nil	0.007	0.004
T. K. R.	M	2:00 p.m.	Before	Nil	0.006	0.003
		4:00 p.m.	After	Nil	0.006	0.003
V. L. B.	F	10:00 a.m.	Before	Nil	0.007	0.004
		3:00 p.m.	After	Nil	0.008	0.004
L. D. G.	F	2:00 p.m.	After	Nil	0.007	0.004
		4:00 p.m.	Before	Nil	0.007	0.004

Nil: no lead was definitely detected beyond the limits of experimental error of the analytical method used.

TABLE 7

Lead content of the blood of healthy males: effect of violent exercise

DESIGNATION	SEX	10 MINUTES OF STAIR CLIMBING	LEAD IN MGM. PER		
			10 grams serum	10 grams cells and fibrin	10 grams whole blood
F. L. S.	M	Before	Nil	0.007	0.004
		After	Nil	0.007	0.004
T. L. W.	M	Before	Nil	0.008	0.004
		After	Nil	0.007	0.004
T. K. R.	M	Before	Nil	0.006	0.003
		After	Nil	0.006	0.003
J. O. G.	M	Before	Nil	0.008	0.005
		After	Nil	0.008	0.005

Nil: no lead was definitely detected beyond the limits of experimental error of the analytical method used.

sequently results of 0.001 mgm. of lead or less may be in error and should be considered qualitatively rather than quantatively. Such a consideration does not change the interpretations drawn from the tables as the important factor is the appearance and disappearance of pathological lead in the serum fraction of the blood.

TABLE 8

Lead content of the blood of healthy females throughout the menstrual cycle

DESIGNATION	CYCLIC INTERVALS	LEAD IN MGM. PER		
		10 grams serum	10 grams cells and fibrin	10 grams whole blood
L. D. G.	Menstruation:			
	First day	Nil	0.006	0.003
	Second day	Nil	0.006	0.003
	End of period	Nil	0.007	0.004
	Quiescent stage	Nil	0.007	0.004
V. L. B.	Menstruation:			
	First day	Nil	0.006	0.003
	End of period	Nil	0.008	0.004
	Quiescent stage	Nil	0.011	0.005
M. V.	Menstruation:			
	First day	Nil	0.007	0.004
	End of period	Nil	0.007	0.004
	Quiescent stage	Nil	0.007	0.004
E. Y.	Menstruation:			
	Third day	Nil	0.006	0.003
	Quiescent stage	Nil	0.006	0.003

Nil: no lead definitely detected beyond the limits of experimental error of the analytical method used.

The manner in which the analysis of the blood of healthy individuals differs from definite clinical cases of acute lead poisoning and periods of exacerbation in chronic lead poisoning, where the symptomatology can not be confused with other diseases, is plainly brought out by a comparison of tables 4 and 5 with table 9. It is evident that the essential difference lies in the appearance of lead in the serum fraction of the blood, since approximately 34 per cent of these cases show a whole blood

TABLE 9

Distribution of lead in the blood during acute lead poisoning and active periods of chronic plumbism

DESIGNATION	SEX	AGE	LEAD IN MGM. PER			CLINICAL MANIFESTATIONS		TYPE OF LEAD POISONING	ETIOLOGY
			10 grams serum	10 grams cells and fibrin	10 grams whole blood	Symptoms according to table 1	Type of hemogram		
A. B.	M	28	0.002	0.010	0.007	Group II	4	Mild acute	Induced
J. dG.	F	37	0.003	0.010	0.007	Group II	4	Mild acute	Induced
J. B.	M	38	0.003	0.041	0.022	Group II	2	Act. chr.	Unknown
A. A.	M	29	0.003	0.023	0.013	Group III	4	Act. chr.	Industrial
V. L.	M	25	0.003	0.018	0.009	Group III	4	Acute	Induced
M. McC.	M	60	0.003	0.073	0.041	Group III	2	Act. chr.	Unknown
J. M.	M	49	0.004	0.009	0.007	Group II	4	Acute	Industrial
A. A.	F	34	0.004	0.014	0.008	Group III	4	Acute	Unknown
P. H.	M	31	0.004	0.014	0.009	Group II	4	Act. chr.	Industrial
E. C.	M	35	0.004	0.016	0.010	Group II	4	Mild act. chr.	Unknown
W. N.	M	62	0.004	0.005	0.005	Group III	4	Act. chr.	Induced
V. L.	M	25	0.004	0.018	0.010	Group III	2	Acute	Induced
A. T.	F	32	0.004	0.008	0.006	Group II	4	Act. chr.	Induced
C. M.	F	3	0.004	0.009	0.006	Group III	4	Acute	Paint
E. C. C.	M	35	0.004	0.016	0.010	Group III	2	Act. chr.	Industrial
H. G.	F	61	0.004	0.023	0.017	Group III	2	Act. chr.	Induced
F. M.	M	58	0.004	0.024	0.018	Group III	4	Acute	Induced
M. P.	F	37	0.005	0.006	0.005	Group II	4	Acute	Unknown
B. A.	F	35	0.005	0.006	0.005	Group II	4	Mild acute	Induced
H. H. H.	M	55	0.005	0.008	0.006	Group II	4	Acute	Unknown
F. G.	F	61	0.005	0.006	0.006	Group III	4	Acute	Induced
J. G.	F	37	0.005	0.011	0.009	Group III	4	Acute	Induced
R. K.	F	52	0.005	0.017	0.011	Group III	4	Acute	Induced
W. H.	M	48	0.006	0.010	0.008	Group III	4	Acute	Industrial
T. Mc.	M	66	0.006	0.010	0.008	Group II	2	Act. chr.	Unknown
W. B.	M	30	0.006	0.010	0.008	Group III	4	Act. chr.	Industrial
A. C.	M	46	0.006	0.016	0.011	Group II	4	Acute	Induced
G. H.	F	57	0.006	0.019	0.011	Group III	4	Acute	Induced
J. B.	M	38	0.006	0.051	0.028	Group III	2	Act. chr.	Industrial
S.	M	25	0.007	0.003	0.005	Group III	4	Acute	Industrial
A. R.	M	48	0.007	0.014	0.011	Group II	4	Acute	Induced
W. R.	M	50	0.007	0.019	0.013	Group III	2	Act. chr.	Induced
M. B.	F	43	0.007	0.026	0.013	Group II	4	Acute	Induced
A. S.	F	50	0.008	0.007	0.008	Group II	2	Act. chr.	Induced
J. G.	M	72	0.008	0.026	0.018	Group II	4	Act. chr.	Unknown
H. S.	M	36	0.009	0.025	0.018	Group III	4	Act. chr.	Industrial
J. DiS.	M	38	0.009	0.024	0.020	Group II	2	Act. chr.	Industrial
J. D.	M	48	0.010	0.009	0.009	Group III	2	Act. chr.	Industrial
H. C.	F	40	0.010	0.011	0.011	Group I	2	Acute	Induced
C. J. V.	M	45	0.015	0.011	0.012	Group III	2	Act. chr.	Industrial
J. M.	M	40	0.015	0.027	0.022	Group II	4	Act. chr.	Induced

Act.: active; Chr.: chronic; Induced: produced by the intravenous injection of lead preparations.

TABLE 10

Distribution of lead in the blood during acute lead poisoning and active period of chronic plumbism with symptoms difficult of clinical diagnosis

DESIGNATION	SEX	AGE	LEAD IN MGM. PER			CLINICAL MANIFESTATIONS		FINAL DIAGNOSIS	ETIOLOGY
			10 grams serum‖	10 grams cells and fibrin	10 grams whole blood	Symptoms according to table 1	Type of hemogram		
R. K.	F	52	0.001	0.003	0.002	Group I	4	Acute*	Induced
A. S.	F	50	0.001	0.006	0.003	Group I	4	Act. chr.*	Unknown
M. B.	F	43	0.001	0.010	0.005	Group I	4	Acute*	Induced
A. F.	M	50	0.001	0.009	0.006	Group II	4	Acute*	Industrial
B. M.	M	35	0.001	0.011	0.006	Group II	4	Acute†	Unknown
F. R.	M	41	0.001	0.017	0.006	Group I	4	Act. chr.*	Induced
W. N.	M	62	0.001	0.014	0.006	Group II	4	Act. chr.*	Industrial
M. J.	F	40	0.001	0.012	0.007	Group I	4	Acute*	Induced
H. G.	F	61	0.001	0.016	0.008	Group I	4	Acute§	Induced
V. L.	M	25	0.001	0.016	0.009	Group I	4	Acute*	Induced
B. A.	F	35	0.001	0.020	0.010	Group I	4	Act. chr.*	Induced
P. D.	M	28	0.001	0.021	0.012	Group II	2	Act. chr.‡	Industrial
W. R.	M	50	0.001	0.037	0.018	Group II	2	Act. chr.*	Induced
T. McC.	M	66	0.002	0.002	0.002	Group I	2	Act. chr.*	Unknown
P. A. S.	M	69	0.002	0.005	0.003	Group II	2	Act. chr.*	Unknown
T. G.	M	26	0.002	0.005	0.004	Group II	4	Acute*	Induced
J. W.	M	60	0.002	0.007	0.004	Group I	2	Acute†	Induced
H. H.	F	40	0.002	0.006	0.004	Group I	4	Acute*	Induced
J. D.	M	38	0.002	0.007	0.005	Group II	4	Act. chr.‡	Industrial
Q. W. O.	M	31	0.002	0.009	0.006	Group I	4	Act. chr.‡	Industrial
E. J.	F	48	0.002	0.020	0.011	Group II	4	Act. chr.*	Induced
S. deR.	F	59	0.002	0.021	0.013	Group II	4	Acute†	Induced
L. C.	F	50	0.002	0.229	0.083	Group II	2	Act. chr.*	Induced
M. R.	M	11	0.002	0.020	0.011	Group I	4	Acute	Unknown
G. B.	M	26	0.003	0.004	0.004	Group I	4	Acute†	X-ray exp.
J. deG.	F	37	0.003	0.010	0.007	Group I	4	Acute*	Unknown
W. R.	M	50	0.003	0.011	0.007	Group II	2	Act. chr.*	Induced
F. K.	M	68	0.003	0.013	0.008	Group I	4	Acute†	Induced
M. G.	F	61	0.003	0.013	0.008	Group I	4	Acute‡	Induced
M. P.	F	37	0.003	0.013	0.009	Group I	4	Acute†	Unknown
L. M.	F	39	0.003	0.019	0.010	Group I	4	Acute*	Induced
E. M.	M	14	0.003	0.002	0.002	Group II	2	Acute†	Paint
L. R.	M	49	0.003	0.015	0.009	Group I	4	Acute*	Induced
B. L.	F	56	0.004	0.021	0.012	Group II	4	Act. chr.†	Induced
E. C. E.	M	35	0.004	0.016	0.010	Group I	4	Act. chr.†	Industrial

* At later period developed definite clinical symptoms.

† Symptoms regressed after calcium and vitamin therapy.

‡ Symptoms became evident upon deleading with acids.

§ After stopping calcium therapy definite symptoms appeared.

‖ The value 0.001 should be considered qualitatively rather than quantitatively.

Chr.: chronic; exp.: experimental; Induced: produced by the intravenous injection of lead preparations.

content of less than 0.010 mgm. lead per 10 ml. of blood and 18 per cent have a whole blood content bordering on the normal range of healthy individuals. However, the fact remains that there is a definite increase in the whole blood values of a large percentage of this group and this is particularly true of acute and subacute episodes during chronic lead poisoning. Although cases of industrial plumbism, as well as those of unknown etiology have been studied, the major portion of our observations have been gained from cases of advanced malignancy during the course of treatment by the intravenous injections of definite amounts of colloidal lead preparations[105]. This enabled us to follow the lead toxicosis through its entire course and permitted a correlation of the blood findings with the symptomatology.

In the preliminary discussion it was shown that it is very difficult to establish a positive diagnosis based on laboratory and clinical findings in cases of latent and early acute phases of chronic plumbism where the patient is apparently free from symptoms other than those of group 1 and the neurological symptoms of group 2, table 1. Thirty-four such cases are reviewed in table 10 and it is shown that there was a definite appearance of lead in the serum fraction of the blood in all the cases although the lead content of the whole blood in 68 per cent was less than 0.010 mgm. lead per 10 grams and 44 per cent fell within the limits of the range established by healthy normal individuals and patients with disorders other than plumbism. All of the cases in table 10 were subsequently proven to be some form of plumbism by one or more of the following procedures: the development of unmistakable symptoms of lead toxicosis when held under observation for a longer period of time, the disappearance of the present symptoms when the patient was placed on calcium and vitamin therapy and in some cases their recurrence when the therapy was stopped, and the development of definite clinical signs and symptoms of plumbism when the patient was placed on acid therapy for divers reasons. In inactive chronic plumbism (table 11) the serum is negative, but there is usually a definite increase in the lead content of the whole blood over that of the control group. This group is

composed of those cases in which a slight metabolic disturbance may cause an acute lead crisis. However, a definite diagnosis may be established in border line cases by placing the patient on acid therapy as practiced in deleading, when an increase in the serum fraction and generally in the whole blood will occur.

TABLE 11

Distribution of lead in the blood during inactive acute and chronic lead poisoning after disappearance of symptoms for four months or more

DESIGNATION	SEX	AGE	LEAD IN MGM. PER			CLINICAL MANIFESTATIONS		ETIOLOGY
			10 grams serum	10 grams cells and fibrin	10 grams whole blood	Present symptoms	Lapse of time since last period of exacerbation	
							months	
L. B.	F	51	Nil	0.014	0.007	None	6	Induced
S. D. L.	F	59	Nil	0.016	0.008	None	4	Induced
L. L.	F	46	Nil	0.017	0.007	None	8	Induced
A. A.	M	29	Nil	0.014	0.008	None	6	Industrial
W. N.	M	62	Nil	0.021	0.009	None	4	Induced
Q. W. O.	M	31	Nil	0.017	0.009	None	7	Industrial
M. B.	F	45	Nil	0.029	0.010	None	6	Unknown
K. V.	M	48	Nil	0.018	0.010	None	4	Industrial
E. C.	M	35	Nil	0.024	0.010	None	6	Unknown
H.	M	32	Nil	0.024	0.010	None	8	Industrial
V. L.	M	25	Nil	0.025	0.010	None	6	Induced
B. J.	M	38	Nil	0.021	0.012	None	11	Induced
H. G.	F	61	Nil	0.034	0.015	None	5	Unknown
W. A.	M	47	Nil	0.022	0.013	None	6	Industrial
T. G.	M	26	Nil	0.031	0.018	None	6	Induced
V. L.	M	25	Nil	0.037	0.020	None	6	Induced
P. B.	F	41	Nil	0.064	0.022	None	7	Unknown
L. C.	F	50	Nil	0.111	0.064	None	2	Induced

Nil: no lead definitely detected beyond the limits of experimental error of the analytical method used.

The mechanism of this procedure is demonstrated in the third case (Q.W.O.) of table 12. A more detailed description of this type of therapeutic procedure will be discussed in a subsequent publication on the treatment of plumbism.

The clinical and physiological course of lead intoxication

TABLE 12

Distribution of lead in the blood during periods of acute exacerbation of plumbism and relation to clinical crisis

DESIGNATION	SEX	LEAD IN MGM. PER			LAPSE OF TIME	SYMPTOMS AT TIME OF ANALYSIS	CLINICAL CRISIS BASED ON		CLINICAL CHART	ETIOLOGY
		10 gram serum	10 gram cells and fibrin	10 gram whole blood			Blood analysis	Symptomatology		
							Day from 1st analysis			
					days					
T. McC.	M	0.002	0.002	0.002	0	Group I	1st	21st	Poor	Unknown
		0.006	0.010	0.008	114	Group II			Died	
A. A.	M	0.003	0.023	0.013	0	Group III	1st	1st	Poor	Industrial
		0.002	0.016	0.010	22	Group II	Lessening		Improved	
		Nil	0.014	0.008	154	Group I	None		Well	
Q. W. O.	M	Nil	0.017	0.009	0	Group I	On calcium		Fair	Industrial
		0.002	0.009	0.006	29	Group II	29th	29th	HCl—27th	
C. P.	F	Nil	0.010	0.005	0	None			Good	Induced
		0.004	0.015	0.010	9	Group I	9th		Fair	
		0.005	0.025	0.014	19	Group II	Peak	19th	Fair	
		Nil	0.022	0.013	36	None			Well	
M. McC.	M	Nil	0.012	0.008	0	Group I			Fair	Unknown
		0.003	0.073	0.041	24	Group II	24th		Poor	
		0.002	0.066	0.044	40	Group III		41st	Died	
O. H.	F	Nil	0.015	0.006	0	None			Good	Induced
		0.002	0.014	0.006	31	Group I	31st		Fair	
		0.003	0.021	0.010	40	Group II		96th	Worse	
		0.005	0.015	0.009	100	Group III			Bad	
		0.009	0.027	0.016	109	Group III	Peak		Bad	
		0.003	0.022	0.011	122	Group I	Lessening		Improved	
		Nil	0.015	0.006	152	None			Well	

B. A.	F	0.001	0.020	0.010	0	Group I	1st		Good	Induced
		0.005	0.006	0.005	24	Group III	Peak	20th	Fair	
		Nil	0.006	0.003	32	Group I			Well	
V. L.	M	0.001	0.016	0.009	0	Group I	1st		Good	Induced
		0.005	0.009	0.007	12	Group II	Peak		Poor	
		0.004	0.018	0.010	19	Group III		19th	Improved	
		0.003	0.017	0.009	74	Group II	Lessening		Better	
		Nil	0.037	0.020	80	None			Well	
		Nil	0.017	0.009	101	Group I			Fair	
		0.003	0.018	0.009	110	Group III	110th	115th	Poor	
		Nil	0.025	0.010	120	Group I	Lessening		Well	
M. B.	F	Nil	0.029	0.010	0	None			Good	Induced
		0.005	0.037	0.017	8	Group II	8th	12th	Poor	
		0.002	0.032	0.012	35	Group I	Lessening		Improved	
		Nil	0.020	0.009	49	None			Well	
H. G.	F	Nil	0.034	0.015	0	None			Good	Induced
		0.004	0.023	0.017	15	Group II	15th	24th	Fair	
		0.002	0.043	0.019	58	Group II	Lessening		Fair	
A. F.	M	0.001	0.009	0.006	0	Group II	1st	1st	Fair	Industrial
		0.002	0.010	0.007	7	Group III	Increasing		Fair	
B. M.	M	0.001	0.011	0.006	0	Group II	1st	1st	Fair	Unknown
		Nil	0.022	0.011	14	None			Good	
		Nil	0.009	0.006	28	None			Well	
J. B.	M	0.003	0.041	0.022	0	Group III	1st	1st	Fair	Industrial
		0.002	0.056	0.030	26	Group II	Lessening		Good	
		Nil	0.042	0.028	33	Group I			Good	
J. dG.	F	0.003	0.010	0.007	0	Group II	1st		Fair	Unknown
		0.005	0.011	0.009	7	Group II	Peak	7th	Poor	
		Nil	0.028	0.014	38	Group I	Lessening		Improved	
		Nil	0.024	0.010	58	None			Well	
E. M.	M	Nil	0.002	0.001	0	None			Poor	Unknown
		0.003	0.002	0.002	6	Group III	6th	12th	Died	

TABLE 12—*Concluded*

DESIGNATION	SEX	LEAD IN MGM. PER			LAPSE OF TIME	SYMPTOMS AT TIME OF ANALYSIS	CLINICAL CRISIS BASED ON		CLINICAL CHART	ETIOLOGY
		10 gram serum	10 gram cells and fibrin	10 gram whole blood			Blood analysis	Symptomatology		
							Day from 1st analysis			
					days					
J. C.	M	Nil	0.002	0.001	0	None			Fair	Induced
		0.002	0.024	0.014	48	Group II	48th	51st	Fair	
		0.002	0.035	0.018	63	Group III			Poor	
		0.005	0.056	0.031	70	Group III	70th	75th	Poor	
		0.005	0.088	0.043	79	Group III	2nd crisis		Worse	
W. W.	M	0.003	0.022	0.011	0	Group III	1st	1st	Fair	Industrial
		Nil	0.018	0.011	7	Group II	Lessening		Fair	
		Nil	0.014	0.008	28	Group I			Good	
		Nil	0.010	0.006	130	None			Well	
I. B.	M	0.002	0.003	0.002	0	Group III	1st	1st	Poor	Induced
J. W.	M	0.002	0.007	0.004	0	Group III	1st	1st	Fair	Induced
J. D.	M	0.002	0.007	0.005	0	Group II	1st	1st	Fair	Industrial
M. S.	F	0.003	0.022	0.012	0	Group III	1st	1st	Poor	Induced
S. K.	F	0.004	0.023	0.014	0	Group III	1st	1st	Poor	Induced
W. N.	M	0.004	0.005	0.005	0	Group III	1st	1st	Fair	Unknown
J. M.	M	0.004	0.009	0.007	0	Group II	1st	1st	Fair	Industrial
F. G.	F	0.005	0.006	0.006	0	Group III	1st	1st	Poor	Induced
J. H.	M	0.005	0.008	0.006	0	Group III	1st	1st	Fair	Induced
L.	M	0.006	0.008	0.007	0	Group II	1st	1st	Fair	Induced
K. L.	M	0.006	0.019	0.011	0	Group III	1st	1st	Fair	Induced
B. G.	F	0.006	0.010	0.007	0	Group III	1st	1st	Fair	Induced
J. G.	M	0.008	0.010	0.009	0	Group III	1st	1st	Poor	Induced
J. DeS.	M	0.009	0.024	0.020	0	Group III	1st	1st	Poor	Industrial
J. D.	M	0.010	0.009	0.009	0	Group III	1st	1st	Poor	Industrial
H. C.	F	0.010	0.011	0.011	0	Group III	1st	1st	Poor	Unknown
S. R.	F	0.012	0.015	0.013	0	Group III	1st	1st	Fair	Induced
B. A.	F	0.014	0.013	0.013	0	Group III	1st	1st	Poor	Unknown
M. T.	F	0.014	0.015	0.014	0	Group III	1st	1st	Poor	Induced
M. R.	M	0.015	0.014	0.014	0	Group II	1st	1st	Fair	Induced
S. V.	M	0.016	0.016	0.017	0	Group III	1st	1st	Poor	Industrial
C. G.	F	0.017	0.020	0.018	0	Group II	1st	1st	Fair	Induced

through its quiescent periods, as well as those of acute and subacute exacerbation may be followed by means of the analysis of the serum fraction and the whole blood for lead, and a prognosis established. Such serial findings are shown in table 12. Realizing that 10 ml. of blood can not be removed from all types of patients as often as desired due to the presence of some other wasting disease, such as malignancy, and having found that there is established a definite relationship between the clinical crisis of plumbism, the serum lead values, (which are usually accompanied by high lead findings in the whole blood especially during active periods of chronic plumbism), and an alteration of the hemographic picture of the peripheral blood, we recommend frequent hemographic study in such patients with subsequent analysis of the blood for lead when indicated after plumbism has been established. Hence, in the discussion of the pathogenesis of this disease it is to be noted that the appearance of lead in the serum fraction of the blood and the increase of lead in the whole blood is attended by a type 4 shift of the neutrophils and hemogram (Crocker) followed by the general symptoms of plumbism. The type 4 shift and hemogram gradually changes over into a type 2 shift and hemogram when the acute period is prolonged or the prognosis becomes critical.

At the approach of death there are indications of a general breakdown of the body's biological and physiological equilibriums accompanied by a flood of lead into the peripheral circulation which is neither eliminated nor detoxified in the manner of the normally functioning defense system. It is shown in table 13 that in cases of latent, mild, or inactive plumbism, the distribution of the lead in the serum, cells and fibrin fraction and whole blood tends to equalize each other at, or shortly before death. This is a relative phenomenon, the absolute quantities of lead present depending upon the type of case. In inactive chronic plumbism and acute periods of exacerbation the same leveling off phenomenon occurs but the actual values of the lead present in the various blood fractions is much higher. Thus a correlation was found to exist between fatalities and the blood crisis. It should be emphasized however

TABLE 13

Distribution of lead in the blood showing the relationship between fatalities and blood crisis

DESIGNATION	SEX	LEAD IN MGM. PER			LAPSE OF TIME BETWEEN ANALYSIS AND DEATH	SYMPTOMS AT TIME OF ANALYSIS	CASE OF DEATH	NECROPSY FINDINGS ATTRIBUTED TO LEAD	LEAD DIAGNOSIS AT TIME OF ANALYSIS	ETIOLOGY
		10 gram serum	10 gram cells and fibrin	10 gram whole blood						
					days					
J. W.	M	0.002	0.007	0.004	74	Group III	Carcinoma	None	Acute	Induced
		0.002	0.003	0.002	14	Group III			Acute	
E. M.	M	0.000	0.002	0.001	11	None	Hodgkin's			
		0.003	0.002	0.002	5	None		None	Acute	Induced
C. M.	F	0.004	0.009	0.007	2	Group I	Plumbism	Positive	Acute	Paint
H. H. H.	M	0.005	0.008	0.006	5	Group II	Plumbism	Positive	Acute	Unknown
T. Mc.	M	0.002	0.002	0.002	121	Group I	Explor. oprtn.		Act. chr.	Unknown
		0.006	0.010	0.008	18	Group II				
K. L.	M	0.000	0.007	0.003	22	None	Carcinoma	None	None	Induced
		0.006	0.019	0.011	15	Group III			Act. chr.	
S.	M	0.007	0.003	0.005	1	Group I	Plumbism	Negative	Acute	Industrial
L. M.	F	0.003	0.019	0.010	12	Group III	Carcinoma	None	Acute	Induced
		0.009	0.017	0.014	1	Group III			Acute	
A. P.	F	0.020	0.029	0.026	1	Group I	Plumbism		Act. chr.	Unknown

A. P.	F	0.020	0.029	0.026	1	Group I	Plumbism			
M. T.	F	0.001	0.002	0.002	162	Group III	Carcinoma	None	Acute	Induced
		0.014	0.015	0.014	1	Group II			Inact. chr.	
C. J. V.	F	0.015	0.011	0.012	10	Group III	Plumbism	Negative	Acute	Industrial
A. H.	F	0.037	0.031	0.035	1	Group II	Leukemia	None	Act. chr.	Unknown
W. A.	M	0.001	0.022	0.013	40	Group I	Carcinoma	Negative	Inact. chr.	Induced
		0.005	0.040	0.024	21	Group III			Act. chr.	
		0.021	0.029	0.026	2	Group II			Act. chr.	
A. dM.	M	0.001	0.020	0.012	40	Group II		Negative	Act. chr.	Unknown
		0.000	0.004	0.002	28	None	Pneumonia		Deleaded	
		0.004	0.094	0.052	1	Group II			Act. chr.	
F. G.	F	0.006	0.103	0.038	2	Group II	Cancer		Act. chr.	Induced
L. C.	F	0.002	0.229	0.083	16	Group II	Carcinoma	None	Act. chr.	Induced
		0.017	0.200	0.106	9	Group III			Act. chr.	

Act.: active; Inact.: inactive; Chr.: chronic.

that the cause of death was seldom proven to be plumbism by necropsy findings.

Since it requires 10 grams of blood to make a determination for lead by the diphenylthiocarbazone method[85], the clinical procedure followed in such cases as are complicated by the presence of malignancy or blood dyscrasia, is to first make an analysis of the lead content of the serum, cells and fibrin fraction and whole blood, then follow the trend of the intoxication by means of daily shiftograms and hemograms[81,82] and make additional blood analyses when hemographically or clinically indicated. However, in uncomplicated cases of plumbism an analysis on 10 ml. of blood is made every second or third day. By such a procedure it is found possible to differentiate between symptoms caused by mild lead poisoning and those which resulted from such pathological conditions as gastro-enteritis, hypercalcemia, food poisonings, sun burns, renal or biliary lithiasis and other causes of colic. This method has proved to be pathognomonic for all types of lead poisoning, whether early, latent, acute, active chronic or inactive plumbism. The therapeutic use of lead can be controlled by this procedure since treatment can be instigated before an acute crisis develops.

SUMMARY

1. A diagnostic procedure was developed for all types of lead intoxication, including incipient, latent, acute and chronic, based upon the lead content of the serum, cells and fibrin fraction and whole blood from patients with some phase of this disease.

2. By this method it is shown that a differentiation could be made between any stage of lead poisoning and other pathological conditions in which the clinical signs and symptoms might be confused with those of lead toxicosis, especially in incipient and latent types.

3. Briefly, the procedure followed on hospitalization consisted of establishing a diagnosis on the basis of the lead findings in the serum, cells and fibrin fraction, and whole blood. In those cases complicated by wasting diseases, the progress of the treatment of the plumbism was followed by daily shiftograms

and hemograms, and making additional lead blood analyses when hemographically or clinically indicated.

4. The onset of acute lead poisoning or acute and subacute periods of exacerbation of chronic lead poisoning can be anticipated and controlled before the clinical crisis develops. Thus, the course of lead toxemia is followed through all its clinical phases, and a basis for the prognosis in this condition established.

5. A relationship between fatalities and lead blood crisis is established, as well as a direct relation between lead blood crisis and clinical symptoms. During the former there is a tendency for the lead in all fractions to assume the same value, the absolute amount depending upon the degree of severity of plumbism encountered, while in the latter there is an increase of lead in the serum fraction usually accompanied by an increase of lead in the whole blood.

6. The possibility of controlling any therapeutic use of lead so as to avoid an acute clinical crisis was suggested, as well as a clinical laboratory method for the control of deleading.

7. A range of lead values for the healthy normal individual is found to be nil per 10 grams of blood serum, 0.002–0.011 mg. per 10 grams of cells and fibrin fraction and 0.001–0.005 mg. per 10 grams of whole blood. This range was independent of sex, age, climatic changes, daily fatigue, violent exercise, meals, menstruation and ovulation. Essentially the same range is established for the blood of patients hospitalized for disorders other than plumbism.

8. A diagnostic procedure pathognomonic for lead in any of its clinical forms and manifestations was developed, based upon the appearance and disappearance of pathological lead in the blood serum, the increase of non-pathological lead in the blood cells and the fluctuation of both in the whole blood.

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Owing to their large number, the list of references is omitted but will be included in the authors' reprints.

MONOCYTIC LEUKEMIA*

With Analysis of Cell Characteristics by Supravital and Fixed Staining Technics

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It is not the purpose of this paper to review the literature nor to discuss the clinical picture of monocytic leukemia but rather to give an analysis of the characteristics of the cells as found in the supravital preparations and by the fixed staining technics, and also the opinions of several pathologists who studied these slides, which tends to point out the difficulties of making a diagnosis.

S. M. F., a male, aged 39, was a patient in Stuart Circle Hospital for a period of eight and one half days. A blood dyscrasia was recognized three or four weeks previously by the attending physician.

The erythrocytic blood picture and the platelet counts are shown in table 1. There was definite evidence of a degenerative erythrocytic blood picture which became quite marked two days before death. There were many normoblasts, many with clover leaf and other bizarre shaped nuclei, Howell-Jolly bodies, erythroblasts showing mitosis and the color index was above one. An average of seven nucleated red cells was noted in differentiating 100 leukocytes. The increase in platelets may be accounted for by the increasing fragmentation of white cells, the fragments being mistaken for platelets, or the total counts given may represent the true state of affairs. Sanford's direct method of counting was used.

In table 2 are shown the white blood cell counts and the differential formulas for the white blood cells. In reference to the differential formulas, after the predominating cells were recognized as monocytes and promonocytes, the blast cells were listed as monoblasts and had the following characteristics which were demonstrated with the Giemsa staining technic. The cytoplasm was dense

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or medium blue with definite mottling and without azurophilic granulations, sometimes showing a suggestion of beginning vacuolation. Many had a perinuclear clear space and condensation of basophilic cytoplasm at the periphery of the cell. The nucleus had purple mottled chromatin, many showing one or not more than two fairly large spherical nucleoli. The nucleoli were well demonstrated by staining the fresh cells with polychrome methylene blue. The nucleus was round or slightly indented or slightly constricted in its middle, was smooth walled, eccentric or centrally placed and formed from eight-tenths

TABLE I

DATE	TOTAL RED CELLS	HEMOGLOBIN	COLOR INDEX	NUCLEATED RED CELLS	PLATELETS
1/28/37	1,210,000	24	1.0	Few	91,000
1/29/37	1,405,000	27	0.96	Few	147,000
2/ 1/37				Many	108,000
2/ 4/37	1,025,000	21	1.05	Very many	85,200
2/ 5/37	840,000	21.5	1.34	Very many	192,000

Volume index 1.04.
Reticulocytes 0.10 per cent.

TABLE 2

DATE	TOTAL WHITE CELLS	DIFFERENTIAL FORMULAE					
		Granulocytes	Lymphocytes	Monocytes	Promonocytes	Monoblasts	Unclassified
1/28/37	38,750	25	15	10	45	5	
1/29/37	47,750	9	6	15	40	27	3
1/30/37	33,500	10	4	19	33	33	1
2/ 1/37	44,000	10	1	23	31	33	2
2/ 2/37	49,500	13	2	24	29	31	1
2/ 3/37	77,500	12	2	32	29	22	3
2/ 4/37	85,200	14	1	25	40	17	3
2/ 5/37	192,000	13	1	34	19	32	1

to nine-tenths of the cell volume. These cells varied in size from 12 to 16 microns. (See fig. 1, cell number 4.)

The young promonocytes averaged 16 microns in diameter. The cytoplasm was gray blue, frequently showing a few fine azurophilic granules, some had from one to many vacuoles. (See fig. 2, cell number 1.) In a few the cytoplasm was completely vacuolated. (See figure 1, cell number 8). There was slight bulging of the edges or one or two pseudopodia showing evidence of having been fixed in the act of motility. (See fig. 1, cells 6, and 7.) The nucleus was large, kidney shaped, oval or round. The shape of some nuclei definitely

suggested having been fixed in the act of motility, as shown in cell number 6 of figure 1. A few cells showed two nuclei. The chromatin was in a very fine sponge-like meshwork.

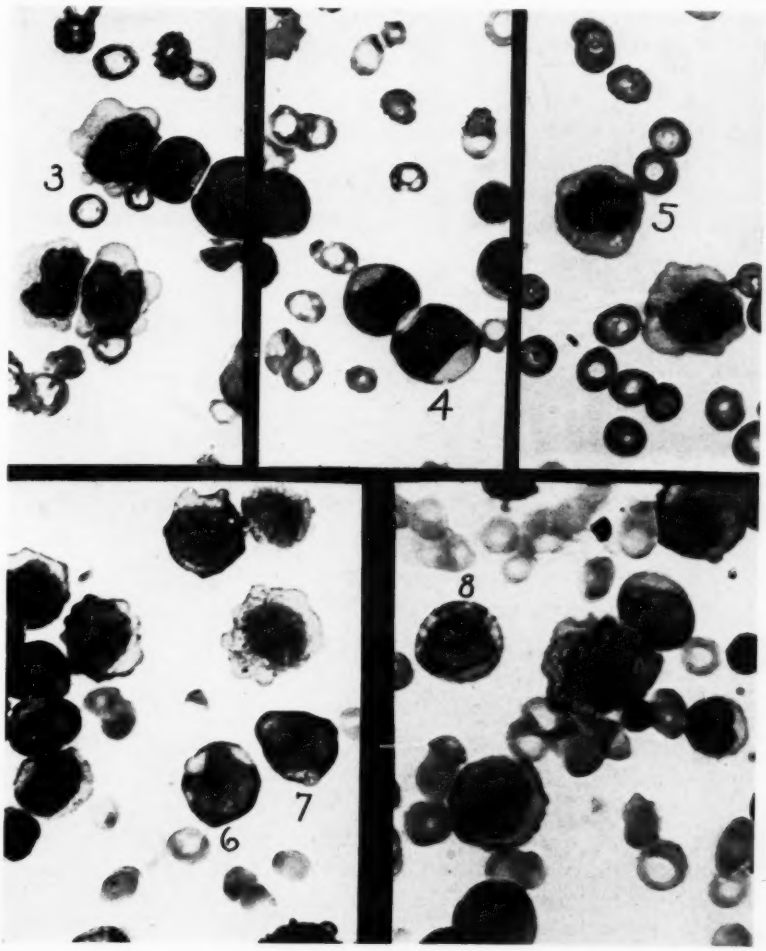

FIG. 1. PHOTOMICROGRAPH OF BLOOD FILMS FROM A CASE OF MONOCYTIC LEUKEMIA. CELLS × 500

The older promonocytes averaged 16 to 20 microns. The cytoplasm was pale blue, had a ground glass appearance and formed two-thirds of the cell volume. There were fine to coarse azurophilic granules dusted over the cyto-

plasm, except at the periphery, the pseudopods usually being free of granules. The outline of these cells was very irregular, being scalloped or having pseudopodia. (See fig. 1, cells 3 and 5.) Many had one or more vacuoles. The nucleus was indented, round, oval or horseshoe shaped, eccentrically placed and rarely smooth walled. The chromatin was both in clumps and in loosely meshed interlacing strands and looked crumpled. It stained a pale reddish purple.

Some monocytes in the films had normal staining and morphological characteristics but were much larger than normal and the azure granules were coarser than those found in normal monocytes.

Many promonocytes presented azur granules that were very coarse and large, some had 8 to 10 large regular round azur granules being as large as the granules

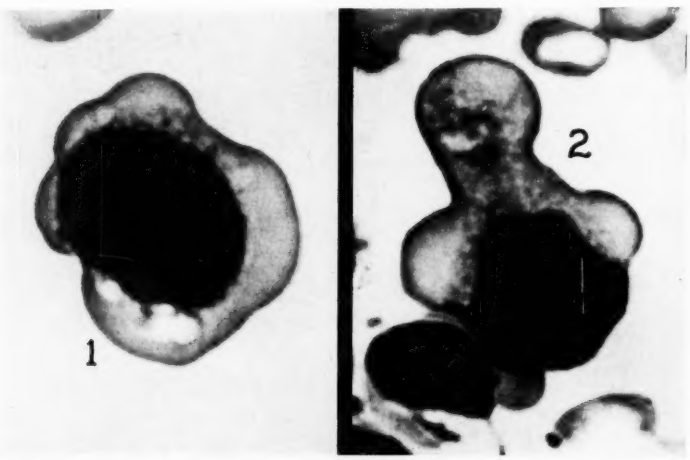

FIG. 2. PHOTOMICROGRAPH OF BLOOD CELLS FROM A CASE OF MONOCYTIC LEUKEMIA. CELLS × 2400

of basophils. Many of these cells had a perinuclear clear space covered with coarse azur granules and a rim of dark blue cytoplasm. The large granules usually stained a very dark red. There was a great variation in the size, shape, distribution and staining intensity of the azur granules. This feature and vacuolation may be looked upon as degenerative changes. The monocytes studied in this case did not show as many vacuoles as are frequently seen, but it seems that this characteristic would vary in monocytic leukemia just as it varies in the neutrophils in different patients having similar toxic conditions.

In monocytic leukemic blood films there is always a liberal sprinkling of granulocytes and some young lymphocytes. Most of the neutrophils in these blood films were without granules, that is, typical agranulocytes. In a differ-

ential count made the day before death the granular cells were classified as follows:

	per cent
Segmented neutrophils with three or more lobes	3
Segmented neutrophils with two lobes	5
Stab neutrophils	4
Metamyelocytes	2
Basophils	1

Because of the lack of granules in the myeloid cells it was impossible to carry the differential any farther to the left, that is, show any greater degree of immaturity. Young myelocytes could not be distinguished from young promonocytes. No doubt a number of the blast cells were myeloblasts but it was impossible to so classify them with any degree of assurance. In the remainder

TABLE 3

DATE	OXIDASE NEGATIVE	OXIDASE POSITIVE	SEGMENTED AGRANULOCYTES	SEGMENTED GRANULOCYTES	SMALL LYMPHOCYTES	MONOCYTES
1/28/37	84	16	10	13	13	64
1/29/37	92	8	8	5	13	74
1/30/37	96	4	14	3	6	77
2/ 1/37	93	7	5	6	10	79
2/ 2/37	96	4	9	2	10	79
2/ 3/37	89	11	8	7	3	82
2/ 4/37	100	0	12		9	79
2/ 5/37	100	0	15		4	81

of the differential counts no attempt was made to classify the myeloid cells, they being all grouped under one heading as granulocytes.

Since erythroblasts showing mitosis and many normoblasts were being crowded out into the circulating blood, it is only reasonable to suppose that young myelocytes and myeloblasts were also being crowded out into the circulation. The myeloid cells were definitely in the minority.

In table 3 is shown the results of oxidase staining. There is considerable confusion in the literature concerning the reaction of the monocyte to the oxidase stain, some authors reporting them as oxidase-positive, others reporting them as oxidase-negative or only faintly positive. The reaction obtained seems to depend on the technic used, and this is rarely given. Graham's benzidine staining technic was used in these studies. In checking this staining technic on a total of 200 monocytes of normal human blood they all were found to be definitely oxidase negative except 9, or 4½ per cent. These did not give a typical oxidase reaction but showed a slight, poorly defined, coarse, irregular stippling or a very few very small rods of dark brown color. This reaction would

not be interpreted as a positive oxidase reaction as a typical reaction with this technic shows large round, distinct and discrete golden brown granules, and is quite typical even in the promyelocytes.

With every preparation stained by the oxidase method, for the studies shown in table 3, a differential count was made of consecutive drops of blood with the Giemsa stain and the two technics compared. The less massive oxidase reaction in the myeloid cells of this patient made the characteristic nuclei plainly visible with the Graham technic. Many agranulocytes were present in the blood films and were oxidase-negative. Each sample of benzidine stain used was tested on normal blood to make certain we could get a good positive reaction. At all times the majority of the cells were oxidase-negative and blood films made the last two days showed all cells oxidase-negative, including the few granulocytes.

The differential cell counts made on the oxidase stained films are not accurate. As is well known, the difficulty in interpreting the type of cell taking an oxidase reaction is great, because the special characteristics by which the cells have been discriminated are not distinctly brought out. With the Graham technic this difficulty is greatly lessened. Mature monocytes show a smudged-like, poorly stained blue nucleus with irregular outline. The very large amount of clear blue cytoplasm usually shows one or more vacuoles more clearly defined than in the Giemsa stain. Cells classified in these differentials as lymphocytes were probably nucleated red cells or dwarf blast cells.

It was impossible to classify the leukemia on the fixed films made the first day as there were only 10 per cent recognizable monocytes. Many of the cells, later classified as promonocytes, could not be distinguished from young lymphocytes or young myelocytes. Supravital studies were begun the second day. Three lots of stain were made for the supravital preparations using 40, 50 and 60 drops of concentrated neutral red stain to 10 cc. of absolute alcohol. The preparation containing 60 drops gave the best results. It was necessary to increase the strength to 70 drops for the last studies made when the total white cell count was 192,000.

It may be difficult or even impossible to distinguish some unusual cells with the supravital technic just as it is with the fixed films, but the difficulties with the supravital method are not always identical with the difficulties with the fixed films, and consequently there are great advantages in a combination of the two methods, checking one against the other. The limitations of the fixed films using any of the blood stains so far devised, are well recognized where the "blast" cells are concerned. It is impossible to definitely distinguish between myeloblasts, lymphoblasts and monoblasts. The trend of the blood picture gives the chief assistance. In the evaluation of the morphological and tinctoral criteria of the supravital technic it may be said that all of the criteria might prove inadequate for identification of the "blast" cells if these cells were the only cell types seen in the preparation. The limitations of both methods must be kept strictly in mind if the correct diagnosis is to be made.

It has been stated that the criteria claimed to be specific for the supravitally stained monocyte, namely, the presence of a rosette of vacuoles in the hof[1] of the nucleus, the type of motility and mitochondrial arrangement, are all variable. I believe, that in a true case of monocytic leukemia (or any other monocytic response), with repeated studies and adjusting the stains to the proper strength, the criteria claimed to be specific can be satisfactorily demonstrated. If the majority of the cells show these criteria, and the other hematologic studies further fortify these findings, the blood dyscrasia can safely be classified as monocytic leukemia.

The supravital preparations were in the microscope incubator and ready to be studied five minutes after they were made. Not that such haste is necessary, but I was desirous of seeing how soon vacuoles would develop and also of noting any change in number and in size. At the end of seven minutes after the preparations were made, one salmon pink vacuole was seen near the hof of the nucleus in a majority of cells averaging 16 to 18 microns. At the end of 12 minutes there was a characteristic rosette of these vacuoles in at least 20 per cent of the cells. (See fig. 3, cell 1.) There was a definite variation in the size of these vacuoles, the ones near the periphery of the cell being largest. These vacuoles definitely increased in size as the preparation stood. This type of cell with a single row of neutral red bodies around the centrosphere in the hof of the nucleus is the youngest promonocyte that can be recognized by the supravital technic and is more easily recognized by this method than by the fixed film methods. These cells did not show any motility that could be recognized as such. This young promonocyte could easily be mistaken for a young lymphocyte or young myelocyte in the fixed film and the peroxidase stain would not clarify the situation since most of the myeloid cells in these films were without granules.

The older promonocytes were a little larger and constituted about 20 per cent of the total white cells. (See fig. 3, cell 2.) At the end of 15 minutes after the preparations were made the cytoplasm opposite the indentation of the nucleus was filled with small salmon pink vacuoles of various sizes. Many of these cells had bulging cytoplasm usually on the same side as the indentation of the nucleus, but they did not have the motility characteristic of that of the monocyte. The vacuoles varied greatly in size, the ones near the periphery of the cells being largest. They definitely increased in size as the preparations were watched, which would eliminate the possibility of their being young myelocytes.

In the Janus green preparations cells, 1, 2, 3 and 4 of fig. 3 showed a large number of small round mitochondria scattered irregularly throughout the cytoplasm. These are not shown in the drawing.

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[1] The "hof" of the nucleus refers to the indentation of the nucleus and signifies "in the portion of the cytoplasm embraced by the indentation of the nucleus."

About 15 per cent of the white cells were observed to have characteristic surface film motility. (See fig. 3, cells 3 and 4 and fig. 4, cells 5, 6 and 7.) The cytoplasm seemed to flow along, having the appearance of a drop of cloudy syrup flowing over a glass slide that is slightly tilted. These cells averaged 20 to 25 microns in their widest part. The vacuoles of cells 3 and 4 of figure 3 showed the characteristic rosette appearance and moderate surface film motility. The vacuoles of cells 5, 6 and 7 of figure 4 were scattered throughout the cytoplasm, varied greatly in size and surface film motility was rather vigorous.

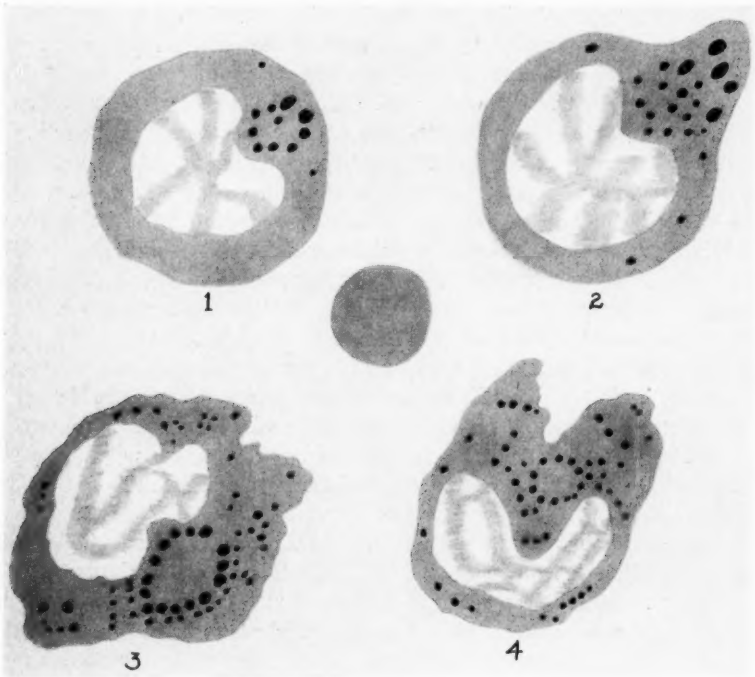

FIG. 3. The large round black bodies, more abundant at the hof of the nucleus, represent the neutral red vacuoles. See text for description.

One of these cells is shown in the fixed film of photomicrograph, figure 2, cell number 2. A number of cells like cell number 8 shown in figure 4 were observed. They averaged 21 microns in diameter, had numerous small, unstained, highly refractive dancing granules which in size and distribution were the same as the azur granules in this type of cell seen in the fixed film. (See fig. 1, cell number 3.) There were a few salmon pink vacuoles which moved about in the cytoplasm. The cytoplasm would roll out in a knob and back again at various points but the cell as a whole was not seen to move. The chromatin of the

nucleus was dense in spots similar to the appearance of the nucleus of this type of cell seen in the stained fixed film.

It was interesting to note that in these preparations the granules of many of the neutrophils did not take the neutral red stain but instead appeared highly refractive, those out of focus appearing black. The motility of the neutrophils was normal.

Supravital preparations studied the day before death showed two or more cells to an oil immersion field which were interpreted as clasmatocytes. See

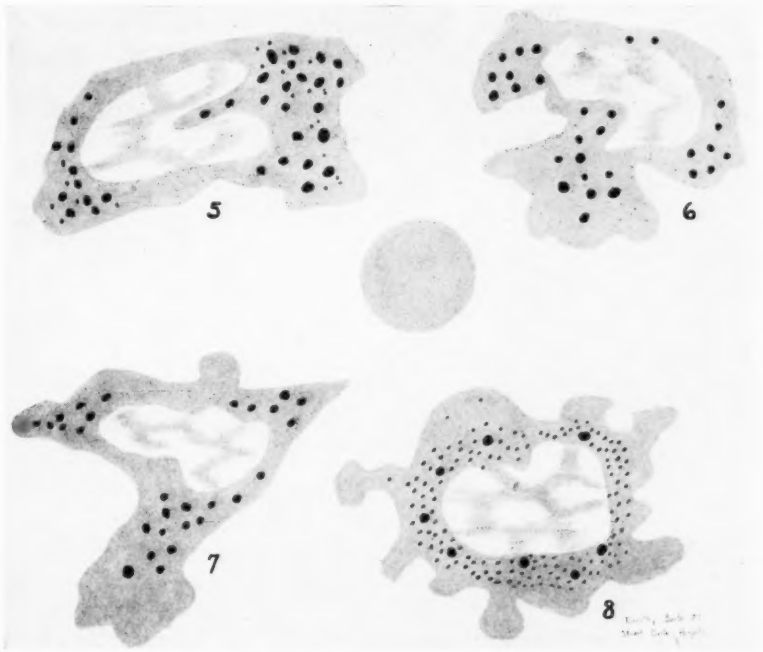

FIG. 4. The large round black bodies scattered irregularly through the cytoplasm of cells 5, 6 and 7 represent the neutral red vacuoles. The small dots in cell 5 represent mitochondria. Cell 8 shows eight neutral red vacuoles, the small dots represent unstained highly refractive granules. See text for description.

figure 5, cell 9. These cells had a rather clear cytoplasm indefinite in outline throughout which many small, various sized neutral red bodies were interspersed. These neutral red bodies varied in color from pink to red and some were yellow. There was no suggestion of a rosette arrangement of the neutral red bodies. Some of the larger neutral red bodies moved about in the cytoplasm. The nucleus was rather indistinct, varied in shape and occupied about one-third of the cell volume. These cells averaged 30 to 40 microns and the

"pseudopods" were approximately 20 to 30 microns in length and could only be seen by careful focusing. In the fixed films there were large smudges interpreted as the remains of these clasmatocytes. Some of them had a pale nucleus averaging 12 microns with fine filaments going out from it in all directions and azur granules over the nucleus and scattered around it. Phagocytosis of red cells by these cells was not noted. Motility was moderately active. The dotted outline in cell 9, figure 5 shows the shape of the cell at the end of drawing.

Supravital preparations made two hours before death showed all of the varieties of white cells previously described and in addition many cells averaging 4 to 6 microns in diameter. (See fig. 5, cell 10.) Some of these were about the color and consistency of red cells, others were grayish-green. They were

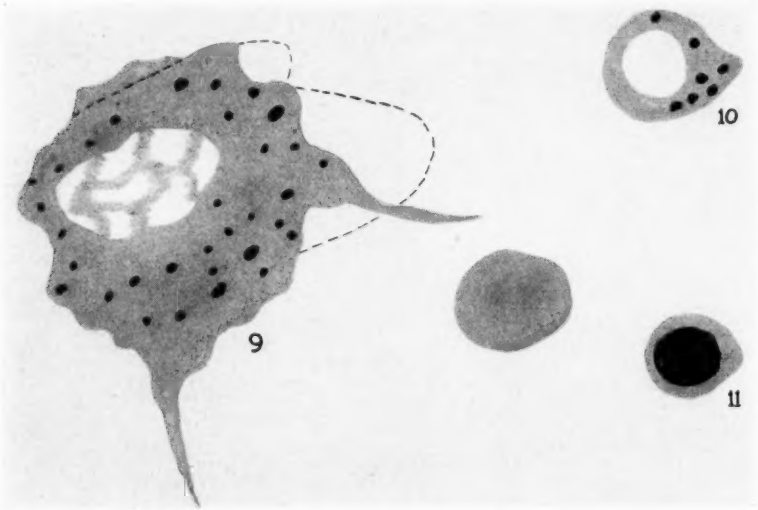

FIG. 5. All of the black structures shown in the cytoplasm of cell 9 represent bodies stained pink, red and yellow by the neutral red stain. For description of cells 9, 10 and 11 see text.

noted 25 minutes after the preparation was made. These cells contained 5 to 10 neutral red bodies that were bright red and many were moving vigorously in the cytoplasm. In some cells these bodies seemed to clump and coalesce after one and one-half hours. Some of these cells had a definite pseudopodic projection. The nucleus was round, colorless and glass-like, and filled three-fourths of the cell. Motility, if present, was very indefinite; some changed position like particles in brownian movement. A few cells like cell 11, figure 5, were observed. The body in the center of the cell was very dense and appeared black. The cytoplasm appeared granular. A number of normoblasts this size, with a similar nucleus, were noted in the stained blood films. It was

impossible to judge if cells with characteristics like those shown for cell 10, figure 5 should be classified as lymphocytes or nucleated red cells. Normal red cells are devoid of intracellular structures that react to the neutral red stain. However, I have observed similar neutral red bodies in the red cells of pneumonia patients who were in coma preceding death.

The postmortem findings disclosed bone marrow cavities filled with tiny bony spicules, very little fat and an abundance of pink marrow. Microscopic study of the autopsy material disclosed the normal marrow replaced by leukemic cells, the architecture of spleen and lymph nodes destroyed by infiltration of leukemic cells and infiltration of leukemic cells into the liver, heart, lungs, kidneys, gastro-intestinal mucosa. As the postmortem microscopic findings do not assist materially in diagnosing the type of leukemia, they will not be discussed. Complete postmortem findings, the case history and clinical course will be reported elsewhere.

DISCUSSION

Is the correct diagnosis monocytic leukemia, an atypical type of myelogenous leukemia, or monocytic leukemia of myelogenous origin?

The blood films and tissue slides from this patient were studied by six pathologists whom I consider experts. Blood films made the first two days the patient was in the hospital were sent to one pathologist and his opinion was as follows: "I believe that a considerable number of the cells are true monocytes but also there are certain findings that would lead me to suspect that these may be myeloblastic, that is, the presence of a considerable number of myelocytes. I believe furthermore, that most cases of monocytic leukemia are in reality temporary variations or atypical forms of myelogenous leukemia. Therefore, a continued study of this case would be very important to determine whether or not it finally eventuated into a myeloid type. I believe that one's concept of such a case as this depends to a considerable extent on one's concept of the origin of blood cells. If I had to put a label on this leukemia I would call it monocytic leukemia of myeloid origin rather than the so-called leukemic reticuloendotheliosis—generalized." This pathologist was then sent blood films made the last two days before the patient's death and also autopsy material. His final opinion was as follows: "I am inclined to believe that this is an atypical case of myelo-

blastic leukemia. The diagnosis of a leukemic process is definite. The question involves the type. It would appear to be of bone marrow origin regardless of what name is applied to it. It seems more likely that this cellular hyperplasia of the marrow has its origin there rather than being infiltrated into the area." Blood films and tissue slides were sent to a pathologist who has made a special study of monocytes. His discussion of the cells is too lengthy to be included but his conclusion was as follows: "In short, I am afraid if I had to make a decision I should have to favor the position of this being a luekemia primarily involving the myeloid strain of cells with a left shift to an extreme degree, and with myeloblasts representing differing degrees of cytoplasmic basophilia . . . I agree with you that there is an occasional cell which is characteristic of the clasmatocyte or desquamated endothelium. However, again this is not an especially helpful sign, because these units are found in leukemic states other than monocytic." This pathologist listed 55 per cent of the cells as myeloblasts for the following reasons: "It has been our experience that the myeloblast more frequently than any other of the primitive white cells, has a tendency to this characteristic condensation of basophilic material at the periphery of the cell with a definite clear halo about the nucleus." However, many cells with these characteristics had typical azur granules, vacuoles, pseudopods or scalloped edges, which characteristics I believe to be due to the peculiar motility of the cytoplasm of these cells. I do not think we can classify the myeloblasts by these characteristics. In fact, with our present staining methods I do not feel that we can definitely distinguish between the blast cells. We can only be guided by the trend of the blood picture. The above pathologist's associate independently concurred in this general interpretation. Another pathologist who is also an expert hematologist examined the blood films and made the diagnosis of "monocytic leukemia Naegeli type," because Naegeli believes that all monocytic leukemias are varieties of myelogenous leukemia. It was his opinion that the monocytes in this case were derived from the myeloblast and he said that it was possible to work out all of the intermediate stages in this process in the blood film.

He further said that many of the cells in the film might be called promonocytes and monoblasts, that their structure was intermediate between myeloblasts and mature monocytes. Two other pathologists who examined the blood films said that the conditions was unquestionably monocytic leukemia.

CONCLUSION

It is not contended that monocytic leukemia cannot be diagnosed without the use of the supravital staining technic but I am of the opinion that in many instances it can be recognized earlier and diagnosed with more assurance if the typical and predominating cells can be demonstrated by both fixed film and supravital methods.

The only basis upon which ultimate differentiation of the several types of leukemia can be accurately and consistently made at the present time is that which rests upon the morphologic and behavior characteristics of the circulating cells themselves.

The diagnosis as to the type of leukemia should be made on the predominating cell found in the circulating blood. One's concept as to the origin of the blood cells is not of importance from a diagnostic point of view.

LYOPHILE COMPLEMENT IN THE KOLMER COMPLEMENT FIXATION TEST FOR SYPHILIS*

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Of the numerous methods proposed for the preservation of guinea pig complement that devised by Flosdorf and Mudd[1] in which complement serum is rapidly frozen and rapidly dehydrated in a vacuum apparatus has proven most satisfactory. Multiple containers of any desired size can be processed to furnish varying amounts of complement, and when dehydration is complete, sealed in vacuo and kept in a refrigerator at a low temperature. For use and to restore the complement, an amount of distilled water corresponding in volume to the amount of original serum is introduced through a rubber stopper with a syringe. The dried material is rapidly soluble, as it remains lyophile, and the complement solution is then ready for dilution, titration and use in exactly the same manner as fresh serum.

There are several advantages attending the use of lyophile complement. In the first place the sera of a large number of guinea pigs can be mixed and processed at the same time, which insures uniform hemolytic activity and fixability of antigen and antibody. In the second place, it permits processing sufficient complement to cover needs for at least 10 to 12 months which saves greatly in time and work as well as proving economical since guinea pigs can be used when the supply is greatest and the cost lowest. In the third place, it permits processing complement during the cooler months of the year when guinea pig complement is apt to be most satisfactory.

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Of course the important question is whether or not lyophile complement preserves not only its hemolytic activity but especially fixability by antigen and antibody, since it is well known that complement preserved by ordinary freezing or with sodium chloride tends to lose first in fixability, sometimes resulting in falsely negative complement fixation reactions.[2] Eagle, Strauss and Steiner,[3] however, have reported that lyophile complement retains its full hemolytic activity for at least eight months in the icebox and in 3477 routine and quantitative Wassermann tests with serum and spinal fluid carried out in duplicate, complement so preserved proved indistinguishable from complement freshly bled and salted for use within a three to four day period. Boerner and Lukens[4] have reported that lyophile complement when stored in a refrigerator at 8° to 10°C. retained its full hemolytic activity and fixability for a period of 12 months. Both the complement and hemolysin titrations remained approximately constant over this entire period. Thereafter deterioration was detected. This complement was used in 12,175 blood and 675 spinal fluid tests and proved in all respects equivalent to fresh complement. On the basis of these results they have discontinued their guinea pig colony because of the greater convenience and uniformity of this type of complement.

On February 7th, 1936, Dr. John Reichel kindly furnished us with a supply of lyophile complement (lot no. 99534) which we kept in a refrigerator at about 8°C. during the day dropping to about 4°C. during the night. At weekly intervals over a period of $4\frac{1}{2}$ months we used this lyophile complement at the same time as fresh complement in complement and hemolysin titrations and for the conduct of Kolmer quantitative complement fixation tests for syphilis.

As shown in table 1, the units of hemolysin with this lyophile complement were practically identical with those observed with fresh complement over the entire period of $4\frac{1}{2}$ months. Furthermore, as shown in the same table, the units of lyophile complement were likewise closely parallel with those of fresh complement so that we were convinced that the lyophile complement preserved its hemolytic activity very satisfactorily over this period of time.

Each week duplicate tests with 13 to 19 sera selected at random from our routine work were conducted with both lyophile and fresh complement totalling 230 sera over the period of 4½ months (table 2). In every instance the results were identical insofar as positive or negative reactions were concerned. Slight quantitative differences however were observed in the degree of complement fixation with positive sera. For example, 11 or 7 per cent of the 157 positive sera gave stronger reactions with lyophile than with fresh complement while 64 or 40.8 per cent of the

TABLE 1

Hemolytic activity of lyophile complement (lot no. 99534) compared with fresh complement

		AGE OF LYOPHILED COMPLEMENT IN WEEKS														
		1	2	3	4	5	6	7	8	9	10	11	12	14	16	18
Hemolysin	1:6000															
	1:5000															
	1:4000	x			x, o	x, o			x	x	x		x, o			x, o
	1:3000	o	x, o	x, o			x, o	x	o	o	o	x, o		x	x, o	
	1:2000							o						o		
	1:1000															
Complement	0.5cc.															
	0.45		o	o				o						o		
	0.4			x	x, o		x, o	x	o	o	x, o			x	x, o	
	0.35	x, o	x			x, o			x	x		x, o	x, o			x, o
	0.3															
	0.25															

x = Unit with lyophiled complement; o = Unit with fresh complement.

157 positive sera gave somewhat stronger reactions with fresh complement. These results indicate therefore that the fixability of lyophile complement may not be quite as good as with fresh complement but as previously stated these differences in fixability were only on the degree of complement fixation in the Kolmer quantitative test since the percentages of positive reactions were identical with both lyophile and fresh complement.

In view of these satisfactory results, we purchased the apparatus in December, 1936, and since then have lyophiled 34 lots of

complement serum. Each lot was a mixture of sera from a large number of healthy adult pigs. The animals were bled at about 11 A.M. and the specimens allowed to stand at room temperature for an hour when the clots were gently broken up and centrifuged. The sera were mixed and kept in a refrigerator until about 2 P.M. when transferred to vials in varying amounts, frozen and dehydrated. After sealing, the vials were kept in a refrigerator

TABLE 2

Comparative fixability of lyophile and fresh complement

AGE OF LYOPHILED COMPLEMENT	NUMBER SERA TESTED	NUMBER POSITIVE LYOPHILED COMPLEMENT	NUMBER POSITIVE FRESH COMPLEMENT	NUMBER STRONGER REACTIONS LYOPHILED COMPLEMENT	NUMBER STRONGER REACTIONS FRESH COMPLEMENT
weeks					
1	19	3	3	0	2
2	15	8	8	0	7
3	15	7	7	0	5
4	15	10	10	0	6
5	15	11	11	0	3
6	14	9	9	1	3
7	18	15	15	4	3
8	13	12	12	2	2
9	15	11	11	0	7
10	16	13	13	0	4
11	15	12	12	0	9
12	15	12	12	2	0
14	15	14	14	2	3
16	15	13	13	0	9
18	15	7	7	0	1
Totals.....	230	157	157	11 (7%)	64 (40.8%)

maintaining a temperature of about 8° to 10°C. during the day and about 4°C. during the night.

Of the 34 lots prepared 4 had to be discarded because of accidents during the processing of the sera. The remaining 30 lots were used at intervals varying from 2 days to as long as 13 months after processing. We observed that in general terms vials of lyophile complement kept on the lowest shelf of our refrigerator were better preserved than those kept on the higher shelves, indicating that the temperature at which lyophiled complement

is kept is a matter of some importance since the lower the temperature the better the preservation.

TABLE 3

Results observed with lyophile complement

LOT NUMBER	INTERVALS USED AFTER PROCESSING	COMPLEMENT UNITS	KOLMER TESTS (TOTALS 7791)
		(*cc. of 1:30*)	
1	3, 15, 17 days; 13 months	0.35–0.45	151; all satisfactory
3	15, 18, 20 days; 13 months	0.35–0.4	159; all satisfactory
4	24, 26 days; 13 months	0.35–0.45	129; all satisfactory
5	2, 4, 15 days; 13 months	0.3 –0.35	159: all satisfactory
6	21, 36, 39, 42 days; 10 months	0.3 –0.35	295; all satisfactory
7	4, 37, 41, 43, 48 days	0.35–0.4	251; all satisfactory
8	47, 49, 63, 68 days; 13 months	0.35–0.4	432; all satisfactory
9	20, 36, 39, 50 days	0.35–0.4	203; all satisfactory
10	3, 31, 36, 42 days; 12 months	0.35–0.4	313; all satisfactory
11	7, 35, 38 days; 9 months	0.35–0.6	255; all satisfactory
12	4, 40, 44, 49, 51 days	0.35–0.5	265; all satisfactory
14	30, 35, 42, 44 days; 11 months	0.3 –0.4	246; all satisfactory
15	36, 57, 60 days; 11 months	0.35–0.4	260; all satisfactory
16	54, 63 days; 7 months	0.35–0.6	224; all satisfactory
17	51, 54, 61, 63, 68 days; 11 months	0.3 –0.4	315; all satisfactory
18	55, 64, 71, 80 days; 11 months	0.3 –0.4	267; all satisfactory
19	56, 68, 96, 100 days; 11 months	0.3 –0.4	297; all satisfactory
20	68, 100 days; 11 months	0.35–0.65	267; unsatisfactory
21	70, 90, 95, 107 days; 11 months	0.45–0.7	342; unsatisfactory
22	76, 81, 83, 123 days; 11 months	0.35–0.45	308; all satisfactory
23	78, 96 days; 12 months	0.5 –0.65	160; all satisfactory
24	90, 99, 112 days; 12 months	0.45–0.65	218; all satisfactory
25	77, 90, 92, 104, 114 days; 12 months	0.4 –0.55	331; all satisfactory
26	74, 78, 94, 100 days; 10 months	0.4 –0.65	374; fair
27	98, 119, 128, 138, 142 days; 10 months	0.4 –0.65	298; fair
28	98, 101, 104 days; 10 months	0.4 –0.65	274; all satisfactory
30	109, 120 days; 10 months	0.4 –0.55	101; fair
32	109, 119, 121, 123, 127, 129, 142 days; 9 months	0.35–0.55	292; all satisfactory
33	74, 81, 84, 86, 88 days; 8 months	0.45–0.5	324; all satisfactory
34	90, 102, 104, 108, 112 days; 9 months	0.45–0.75	281; all satisfactory

Table 3 shows the intervals or age of the different lots of lyophile complement used; also the variation in the complement titrations and the results observed with 7791 routine Kolmer quantitative complement fixation tests for syphilis with sera

and spinal fluids which were conducted at the intervals shown after processing the complement. Fresh complement was not employed as we depended solely upon the lyophile complement in the conduct of our routine tests.

In some instances the complement units were higher than we have learned by experience to expect in the case of fresh complement but the complement fixation reactions were highly satisfactory with 25 of the 30 lots, fair with 3 and unsatisfactory with two. By "fair" we mean that the lyophile complement was slightly more susceptible to the anticomplementary effects of antigen alone and some of the sera alone than perfectly satis-

TABLE 4

Summary of results observed with lyophile complement 2 days to 13 months after processing

NUMBER LOTS	AGE OF LOTS	RESULTS OF KOLMER QUANTITATIVE REACTIONS
3	4 to 51 days	All satisfactory
1	54 days to 7 months	All satisfactory
1	74 days to 8 months	All satisfactory
3	7 days to 9 months	All satisfactory
5	21 days to 10 months	2 sets satisfactory; 3 fair
8	30 days to 11 months	6 sets satisfactory; 2 unsatisfactory
4	3 days to 12 months	All satisfactory
5	2 days to 13 months	All satisfactory

factory complement, while "unsatisfactory" means that the complement was so sensitive to antigen alone and sera alone as to result in a large percentage of unsatisfactory reactions.

These results are summarized in table 4 and indicate that lyophile complement kept for about 10 months begins to become unsatisfactory although 9 lots tested at intervals of 2 days to as long as 12 to 13 months after processing were still quite satisfactory.

CONCLUSIONS

1. Complement dehydrated from the frozen state and sealed *in vacuo* ("lyophile complement"), when stored in a refrigerator at 4° to 10°C. retained a satisfactory hemolytic activity and

fixability for at least 10 months and 9 lots were perfectly satisfactory up to as long as 12 to 13 months after processing. Longer periods of preservation have not been tested.

2. The 30 lots of lyophile complement were used in 7791 Kolmer quantitative complement fixation tests with sera and spinal fluids for syphilis at intervals varying from 2 days to 13 months after processing. Of these the results were very satisfactory with 25 lots or 83 per cent, fair with 3 or 10 per cent and unsatisfactory with 2 or 7 per cent.

3. Under the circumstances we regard lyophile complement kept at 4° to 10°C. as quite satisfactory and highly advantageous for the conduct of complement fixation tests for syphilis for at least 10 months after processing and probably for at least 13 months.

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FLOCCULATE INDUCED ANTIBODIES AND SYPHILIS IMMUNITY IN RABBITS*

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Various biological substances have been shown to cause positive syphilis tests in rabbits when inoculated intravenously. Eagle,[1] by means of complement fixation tests, has shown that flocculate from human syphilitic serum will increase the reacting substance in the serum of rabbits when injected intravenously. Later it was demonstrated by Rytz[2] that flocculate from the serum of a rabbit thus inoculated will give rise to identical antibodies in another rabbit when administered intravenously, and that the total amount of serum from an immunized rabbit contains many more units of flocculate than were originally injected. This would seem to indicate that the tissue cells are specifically activated by introduction of the flocculate.

Torii[3] produced positive syphilis reactions in rabbits by intravenous injections of an aqueous emulsion of egg yolk; and also was able to show that a lipoid extract from syphilitic rabbit testicles will cause positive Wassermann tests in rabbits when such material is injected intravenously, and that lipoid extract from normal rabbit testicles failed to induce antibodies able of causing positive syphilis tests in rabbits. He further showed that a protein emulsion from syphilitic rabbit testicles does not cause positive reactions when injected in the same manner. Treponema pallida vaccine from a culture was shown by Kertesz[4] to produce partial immunity to syphilis when used intravenously in rabbits and such material was also found to be of therapeutic value in rabbit syphilis.

Mesentery extract was found by Bergel[5] to contain anti-

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syphilitic substances. Rabbits inoculated both locally and intravenously with such extract showed a high degree of immunity to treponema pallida when intratesticularly injected. The same extract was also found to possess therapeutic properties. Treponema pallida, when treated in vitro with mesentery extract, was seen to undergo disintegration, although a few organisms apparently resisted anti-substances of that nature. When lipoids such as lecithin were injected into the peritoneal cavity of rabbits prior to intratesticular inoculations with treponema pallida, the incubation period was shortened considerably, as typical lesions would appear within 11–12 days. In rabbits not lecithin treated, the incubation period was 3–4 weeks. On the other hand, if rabbits were given preliminary intraperitoneal injections with treponema pallida, and 3 days later inoculated intratesticularly with the same organism, the primary lesions in the testicles were delayed 3–4 months.

The above experiments seem to suggest the existence of both specific and nonspecific anti-syphilitic biological substances.

SEROLOGIC TESTS IN EXPERIMENTAL SYPHILIS

It is well known that some serodiagnostic tests for syphilis give positive results on serum from normal rabbits. Eagle[1] found the Wassermann positive in about 50 per cent of normal rabbits, and Porro,[6] who made an extensive investigation on blood from various species of animals with the Kahn test, showed that reaction to be positive in 80 per cent of a small number of rabbits tested. However, in 42 white New Zealand rabbits the present author found the Kahn test positive in only 2 instances. Most complement fixation methods are unreliable in experimental syphilis because the procedure is more or less nonspecific on rabbit serum, or too many samples of blood from that animal are found anticomplementary.

In the present work, the Rytz flocculation methods[7,8] were used exclusively, as such procedure proved to give constantly negative reactions on serum or whole blood from normal rabbits not inoculated with flocculate or treponema pallida. A total of 98 normal rabbits of various breeds were tested and found negative by these flocculation methods.

THE FORMATION OF ANTIBODIES IN RABBITS BY INOCULATION OF FLOCCULATE FROM HUMAN SYPHILITIC SERUM

The flocculate as obtained from human syphilitic serum by the flocculation method was washed once in distilled water, then shaken into a fine emulsion in normal saline. It was found that doses of 16 units of flocculate were as effective as 90 units used in earlier experiments.[2]

Forty normal white New Zealand rabbits with negative flocculation tests were each injected intravenously every fifth day for 3 weeks with 16 units of flocculate (flocculate from four 4 plus reactions) from human syphilitic serum. Five days after the second inoculation all animals showed positive flocculation reactions, and 8 days after the fourth injection all tests were strongly positive. Six rabbits were given flocculate which had been heated in a water bath at 60°C. for 1 hour. A week after the fourth inoculation these animals proved to have negative flocculation tests. Three rabbits were then injected in the same manner with flocculate that had been heated in a water bath at 56°C. for 30 minutes; all of these animals showed strongly positive tests a week after the fourth inoculation.

Nearly all rabbits inoculated with flocculate from human syphilitic serum appeared to be in much better physical condition than rabbits not treated with such material. The inoculated animals showed more alertness and activity and also increased more rapidly in weight than the untreated rabbits. However, among the treated as well as among the untreated animals a few deaths occurred, mostly due to "snuffles." The rabbits inoculated with unheated flocculate or with flocculate not heated above 56°C. for 30 minutes showed more or less positive flocculation tests for about 1 year.

ANTIBODIES INDUCED BY INOCULATION OF NONSPECIFIC FLOCCULATE FROM CASES WITH MALARIA

Blood was obtained from 2 presumably nonsyphilitic patients with malaria at the Minneapolis General Hospital. The serum from these patients showed moderately strong positive flocculation reactions, and the flocculate thus obtained was prepared in the usual way for intravenous inoculations of 2 rabbits. After 4 inoculations at 5 day intervals both animals proved to have very strongly positive flocculation tests a week after the last inoculations. In contrast to the increased physical well-being of the animals injected with flocculate from human syphilitic serum, the rabbits inoculated with the material from the serum of patients with malaria, became marasmic and died a few weeks following the inoculations. An examination of stained blood smears from these animals a few days before they died showed a marked anisocytosis with few normoblasts and slight polychromatophilia.

RESULTS WITH FLOCCULATE FROM THE SERUM OF PATIENTS WITH LEPROSY

Through the courtesy of the United States Hospital, Carville, Louisiana, 12 blood samples were obtained from presumably nonsyphilitic patients with

leprosy who were known to have positive flocculation tests for syphilis. The material proved sufficient only for 4 flocculate injections of 1 rabbit, and in that animal the flocculate from such serum failed to induce a reacting substance which could be demonstrated by Rytz or Kahn reactions. The test animal remained alive and well.

FLOCCULATE OBTAINED FROM COW SERUM

Pooled cow serum as secured from the packing plants for Loeffler's media, uniformly gives positive flocculation tests by the various methods including the procedure employed in this work. Four rabbits were injected intravenously with such flocculate, each given 4 times 16 units. A week after the last inoculations all rabbits showed strongly positive flocculation reactions. Those animals also remained positive for about 1 year.

THE PROPHYLACTIC AND THERAPEUTIC VALUE OF FLOCCULATE FROM HUMAN SYPHILITIC SERUM IN SYPHILITIC RABBITS

Through the courtesy and the co-operation of the United States Laboratory, Long Island, New York, syphilitic Chinchilla rabbits were secured from that laboratory for the purpose of transferring that strain of treponema pallida to white New Zealand rabbits. The chancrous testicles of an infected rabbit were emulsified in saline at body temperature. The emulsion was then injected into the testicles of 6 New Zealand rabbits, and also inoculated into the testicles of 6 rabbits of the same strain previously inoculated intravenously with flocculate from human syphilitic serum. In 4 of the 6 animals of the first group (injected with treponema pallida only) typical lesions appeared in the testicles 3–5 weeks after introduction of the organisms. The fifth rabbit showed an atypical lesion, but the 5 animals developed positive flocculation tests at the same time. One rabbit showed no lesions, and the flocculation test remained negative. About 10 days after the flocculation tests had become positive, the testicles showing lesions were removed, and the presence of typical treponema pallida demonstrated by dark-field examinations.

In the 6 rabbits that prior to the intratesticular inoculations with treponema pallida had been injected intravenously with flocculate from human syphilitic serum, the incubation period was considerably prolonged as no primary lesion appeared before 12–16 weeks after inoculation of the treponema pallida, and in all of the 6 animals the lesions were smaller and atypically developed compared with the lesions of the former group not injected with flocculate. After removal of the testicles, the darkfield examinations revealed few typical treponema pallida organisms. Numerous long, straight rods were seen moving very sluggishly across the field. Also the few morphologically typical treponema pallida present were sluggish in their movements. Similar results have been obtained by Becker[9] on a larger series of rabbits.

Of the rabbits injected with flocculate prior to the treponema pallida inocu-

lations, only 1 developed secondary lesions, and of the animals not treated with flocculate, 3, of which 1 died early, developed secondary symptoms. The remaining 2 rabbits with secondary lesions gradually became marasmic, and the back, neck and ears were covered with open lesions. The eyes looked watery and inflamed. At that stage the flocculation tests had become negative or doubtful respectively. The 2 animals were then given intravenous inoculations of flocculate from human syphilitic serum. After 3 injections the lesions had practically healed; the animals gained in weight and became alert and active, and the flocculation tests again became positive due to introduction of the flocculate.

POSITIVE FLOCCULATION TESTS IN NORMAL RABBITS INJECTED WITH FLOCCULATE FROM THE SERUM OF SYPHILITIC RABBITS

From time to time, serum was obtained from 2 syphilitic rabbits that prior to the inoculations of treponema pallida had not been injected with flocculate. From that serum, flocculate was prepared in the usual manner for the inoculation of 3 normal rabbits. A week after the fourth injection the 3 animals showed weakly positive flocculation tests, and after 8 inoculations the tests proved to be strongly positive. Injections were then discontinued for 4 weeks at which time the titre of the reacting substance apparently had decreased, but after 2 injections at that time with the same type of flocculate, the reactions became strongly positive. The 3 animals were then given 2 weekly intravenous injections of 1 cc. of 3 per cent neoarsphenamine. As expected, the chemical treatments had no apparent influence on the positiveness of the flocculation tests.

COMMENT

There is hardly a bacterial disease in which the antibacterial substance is more abundant and so constantly present than in syphilitic infection. Nevertheless, the true nature of the material causing changes in syphilitic serum is but little understood. There is still room for skepticism regarding the protective ability of the reacting matter demonstrable through diagnostic tests for syphilis. The results described above are only suggestive of the protective rôle of such reacting indicative substance. Actual proof would call for considerably more and deeper investigation.

In the above connection it should be kept in mind that the presumed antiagent employed, in a strict immunological sense, can be described as only semispecific as the treponema pallida it was used against had been grown in rabbit tissue for a number

of years and was therefore supposedly nonvirulent to human beings. To get the experiment on a strict specific basis, flocculate from the serum of syphilitic rabbits should be employed in syphilis of that animal to test the specific protective nature of syphilitic antibody as demonstrated through flocculation reactions.

SUMMARY

It has been shown that the Rytz flocculation methods for the diagnosis of syphilis constantly gave negative reactions on blood from normal rabbits, and that flocculate obtained by those methods from human syphilitic serum, and also from the serum of syphilitic rabbits, will induce antibodies in rabbits demonstrable by flocculation tests. Also, nonspecific flocculate from cow serum and from patients with malaria, when injected intravenously, will give rise to antibodies in rabbits detectable by the same reaction. In a single experiment, the flocculate from the serum of several patients with leprosy failed, when inoculated intravenously, to induce antibodies in a rabbit which could be demonstrated by syphilis tests.

The physical well-being of rabbits inoculated intravenously with flocculate from human syphilitic serum has been noted. It has also been shown that rabbits injected intravenously with flocculate from patients with malaria died from marasmus, and that the blood morphology in those animals indicated anaemia. The prophylactic and therapeutic value of flocculate from human syphilitic serum in syphilitic rabbits has been discussed.

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SERUM CHOLESTEROL FLUCTUATIONS DURING THE MENSTRUAL CYCLE*

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For many years medicine has been aware of the close chemical relationship between the female sex hormones and the lipin sterols physiological to man. Although there is no direct evidence of the interconversion between sterols and hormones, there have been many studies of the possible mechanisms involved. Another type of investigation dealing with these relationships was that originally presented by Bloor, Okey and Corner.[1] These authors, by following the lipid content of sow corpora lutea in correlation with the state of physiological activity known to hold for the given specimens at the time of abstraction, were able to show that such heightened activity was accompanied by increasing amounts of free cholesterol and phospholipids along with a decreasing ester cholesterol. A great deal of work was subsequently done by Boyd[2,3] who definitely confirmed the findings of his predecessors. In addition, Boyd clarified certain mechanisms of ovarian function with regard to true and pseudo-pregnancy (in rabbits), by using the above type of lipoid fluctuations as an index of physiological behaviour. Building upon this background, Boyd[4] found that, while the cholesterol ester of the corpus luteum did not vary with the age of the tissue, the free cholesterol rose steadily from the time of follicular rupture to the albicans stage. Simultaneously, the corpus luteum hormone has its greatest concentration just following the inception of the corpus luteum, and then declines steadily to the albicans stage. These facts suggest an inverse relationship

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between the luteum hormone (or progestin) and free cholesterol. Corner[5] states that the end product of the corpus luteum is a mass of tissue with large amounts of glycerides and free cholesterol, as a result of fatty degeneration. Menstruation supposedly follows within 48 hours of this retrogression.

Sensing the importance of the cholesterol: female sex hormone relationships, Okey and Boyden[6] published a very careful study of the blood lipid levels during the various phases of the menstrual cycle. Their review of the prior literature cites the paucity of similar studies. These authors, using 26 monthly cycles involving 200 fasting blood specimens, found the most striking cyclic alteration to be a fall in total blood cholesterol, "which took place almost invariably during or within a few days of the menstrual period." This was "usually preceded or followed by blood cholesterol levels higher than the average for the individuals concerned." If the average of the observed values for each individual be taken as 100 per cent, and each observed value be computed in terms of this average, the high points of the curve come at 124 per cent while the average of the low values observed is 70 per cent, a variation of 54 per cent of the so-called normal value for each individual.

Approaching this problem of cholesterol fluctuation within the menstrual cycle, it would seem that the need of ascertaining the type of such physiologically normal changes is paramount before proceeding to pathological deviations. Normal values have long been set by statistical analysis of a large series of determinations made on fasting blood specimens, taken at random on so-called healthy subjects. In the case of women, distinction is not usually made from the male levels, and no effort is usually made to compensate for menstrual aberrations. That there are both points of needless labor and errors of omission in such a procedure, have recently been proved. Okey and Stewart[7] showed that diets high in cholesterol, although possibly elevating the blood plasma levels after long continued ingestion, had no such effect after short periods. Feraru and Offenkrantz[8] using medical students, demonstrated the failure of normal diets to influence the post-absorptive blood cholesterol levels when com-

pared to fasting values. Boyd[9] concludes that "normal individuals under normal conditions of life, and ingesting three normal meals a day, have been shown to exhibit but slight (diurnal) variations in concentration of plasma lipids." Other workers now agree that the old dictum of "use fasting blood specimens" need not apply for cholesterol determinations.

Offenkrantz and Karshan,[10] in ascertaining the so-called normal values for children, found the statistical mean for their series to be somewhat lower than that for adults, although the total range of findings was as large. Of greater interest is the fact that the values for pre-pubescent boys and girls did not differ notably from each other. Likewise these authors found[11] that young female rheumatic fever patients, studied by weekly determinations over a long period of time, showed no cyclical variations such as their adult sisters will be found to exhibit.

Schube,[12] continuing this search for the physiological norm (or the lack of it), found that there seemed to be no definite pattern along which the total blood cholesterol levels fluctuated, but these values did vary from week to week as much as 73 mg. per cent. In this work, as well as in all others noted here, the validity of the actual techniques are considered beyond question.

On the positive side of this newer investigation is the fractionation of the total serum cholesterol into esterified and non-esterified portions. It was definitely shown by Sperry[13] that, whereas the total serum cholesterol may fluctuate greatly within a group, the percentage of free cholesterol (of any total value) will be close to a mean of 26.9 per cent. All of our work has tended to confirm this observation. Finally there is a newer consideration, rapidly becoming a certainty, that cholesterol levels in the blood of healthy individuals are, among other considerations, a product of the constitutional: psychiatric makeup. Using definite constitutional and personality types from among close male associates, we[14] have shown definitely lower cholesterol levels to be maintained by the tall, slender, asthenic group, as compared to the higher levels of the short, thick set, sthenic group. Scube,[15] and Gildae et al.[16] have shown that a difference in blood cholesterol levels exists in the case of pathological psychi-

TABLE 1

SUBJECT	MONTH	DAY	FREE CHOLESTEROL	TOTAL CHOLESTEROL	FREE CHOLESTEROL	
			mgm. per cent	*mgm. per cent*	*per cent*	
No. 1*	11	16	58.0	192.2	30.2	
24 years old	11	20	51.0	190.5	26.8	
Sthenic habitus†	11	25	51.1	188.6	27.1	
	11	26	53.8	173.7	30.9	Menses
	12	1	57.9	208.4	22.9	
	12	6	45.7	184.4	24.8	
	12	11	50.0	185.9	26.9	
	12	15	52.6	176.8	29.8	
No. 2*						
	11	21	59.8	193.2	32.3	Menses
25 years old	11	26	62.9	211.6	29.7	
Athletic habitus	11	29	54.2	173.9	31.1	
	12	6	57.9	185.0	31.3	
	12	10	51.7	170.0	30.4	
	12	13	55.8	194.0	28.8	Menses
	12	16	54.3	199.8	27.2	
	12	21	57.2	190.1	29.7	
No. 3*	11	21	41.2	140.0	29.4	
21 years old	11	26	42.5	141.4	30.1	
Asthenic habitus	11	29	37.0	135.6	27.3	
	12	2	37.6	118.2	31.8	Menses
	12	6	33.4	142.2	23.4	
	12	10	36.7	140.6	26.1	
	12	15	39.2	129.9	30.2	
	12	21	43.9	133.4	32.9	
No. 4*	11	21	59.6	188.6	31.6	
21 years old	11	26	50.1	168.4	29.8	
Asthenic habitus	11	30	45.4	152.3	29.8	Menses
	12	4	56.2	226.7	24.8	
	12	10	43.1	170.5	25.3	
	12	14	52.8	177.1	29.8	
	12	21	56.2	181.8	30.9	
No. 5*	11	21	49.8	170.0	29.3	
27 years old	11	26	45.0	162.0	27.8	
Asthenic habitus	11	29	47.0	155.3	30.3	Menses
	12	2	50.7	175.0	26.0	
	12	7	45.5	155.3	28.5	
	12	11	50.1	172.2	29.1	
	12	15	51.3	172.2	29.8	

* Indicates subjects on night nursing duty.

† The terms asthenic, sthenic, and athletic are used here to indicate constitutional types: Asthenic, tall, slender type. Sthenic, short, usually heavily proportioned type. Athletic, intermediary type,—generally tall, well built type.

TABLE 1—*Continued*

SUBJECT	MONTH	DAY	FREE CHOLESTEROL	TOTAL CHOLESTEROL	FREE CHOLESTEROL	
			mgm. per cent	*mgm. per cent*	*per cent*	
No. 6	10	18	57.3	204.0	28.6	
30 years old	10	21	54.3	200.3	27.1	
Athletic habitus	10	27	54.7	192.7	28.4	
	11	1	54.4	184.6	29.5	Menses
	11	4	43.9	189.3	23.2	
	11	9	46.2	179.9	25.7	
	11	14	52.2	188.4	27.7	
	11	18	56.3	198.9	28.3	
No. 7	1	3	63.9	218.1	29.3	
19 years old	1	7	58.8	208.4	28.3	
Sthenic habitus	1	10	57.1	190.3	29.9	Menses
	1	13	61.5	223.6	27.5	
	1	20	62.3	217.0	28.7	
	1	24	62.1	214.0	28.9	
	1	29	58.2	196.7	29.6	
	2	2	63.6	212.2	30.0	
No. 8	1	3	54.6	174.0	31.4	
21 years old	1	6	57.5	148.6	32.2	Menses
Sthenic habitus	1	10	51.8	192.1	26.9	
	1	15	49.7	183.4	27.1	
	1	20	52.9	188.2	28.1	
	1	24	53.9	188.7	28.6	
	1	28	53.3	190.5	27.9	
	2	1	55.2	179.3	30.8	
No. 9	1	7	60.4	197.3	30.6	Menses
26 years old	1	10	70.1	246.7	28.4	
Sthenic habitus	1	13	77.5	249.1	31.1	
	1	17	61.9	219.0	28.3	
	1	21	68.1	236.0	28.7	
	1	26	62.4	214.6	29.1	
	1	30	62.1	217.3	28.6	
	2	4	58.9	195.2	30.2	
No. 10	7	14	41.1	157.6	26.1	
19 years old	7	18	39.2	153.4	25.8	
Asthenic habitus	7	21	37.4	148.1	25.3	
	7	26	37.1	139.7	26.6	Menses
	7	30	39.1	160.5	24.4	
	8	4	35.1	154.9	22.7	
	8	8	37.2	158.4	23.5	
	8	12	37.7	151.2	25.0	

TABLE 1—*Continued*

SUBJECT	MONTH	DAY	FREE CHOLESTEROL	TOTAL CHOLESTEROL	FREE CHOLESTEROL	
			mgm. per cent	*mgm. per cent*	*per cent*	
No. 11	7	9	71.8	238.6	30.1	
28 years old	7	13	70.3	231.3	30.4	
Athletic habitus	7	17	66.0	218.7	29.8	
	7	20	70.9	224.5	31.6	
	7	23	69.4	225.1	30.9	Menses
	7	28	69.7	247.2	28.2	
	8	3	78.9	246.8	31.9	
	8	7	70.6	233.0	30.3	
No. 12	7	8	58.4	212.4	27.5	
22 years old	7	12	61.7	218.1	28.3	
Sthenic habitus	7	15	57.9	221.0	26.2	
	7	19	43.1	209.6	25.7	
	7	25	56.1	191.0	29.4	Menses
	7	28	65.3	227.0	28.8	
	8	2	57.6	198.7	29.0	
	8	5	59.6	204.1	29.2	
No. 13	7	4	47.4	196.0	24.2	Last
21 years old	7	8	50.6	179.9	28.1	menses
Asthenic habitus	7	12	45.0	173.4	26.1	
	7	16	54.3	184.0	29.5	
	7	21	52.2	180.2	28.9	
	7	24	45.7	169.0	27.0	
	7	29	48.1	173.6	27.7	
	7	31	47.9	165.3	29.6	Menses
	8	3	47.9	191.8	25.0	
No. 14	2	22	41.4	160.0	25.9	
27 years old	2	25	39.7	154.4	25.7	
Athletic habitus	3	1	38.1	153.1	24.9	
	3	7	38.0	148.6	25.6	Menses
	3	11	48.5	169.3	28.6	
	3	15	38.0	150.7	27.2	
	3	18	40.2	147.7	27.3	
	3	21	40.7	155.9	26.1	
No. 15	11	17	53.7	186.7	28.8	
20 years old	11	20	57.3	193.5	29.6	
Asthenic habitus	11	24	56.5	188.2	30.0	
	11	29	57.4	190.4	30.1	
	12	4	51.1	186.1	27.5	
	12	8	47.5	181.4	26.2	
	12	10	48.4	179.5	27.0	Menses
	12	13	50.4	194.0	26.0	

TABLE 1—*Concluded*

SUBJECT	MONTH	DAY	FREE CHOLESTEROL	TOTAL CHOLESTEROL	FREE CHOLESTEROL	
			mgm. per cent	*mgm. per cent*	*per cent*	
No. 16	11	3	59.8	230.3	28.6	
29 years old	11	7	64.2	230.0	27.9	
Sthenic habitus	11	10	62.3	221.6	28.1	Menses
	11	14	64.6	240.4	26.8	
	11	19	61.1	240.5	25.4	
	11	25	61.4	238.0	25.8	
	11	29	65.3	240.1	27.2	
	12	2	65.0	234.8	27.7	

atric types which are considered to derive from the above constitutional patterns. Reports of this type, although momentarily adding to the confusion surrounding blood chemistry levels, must eventually clarify the mechanisms involved.

PROCEDURE AND FINDINGS

The material used in this study consisted of about 8 cholesterol determinations for each of a complete menstrual cycle on 16 women. In an effort to eliminate all possible secondary factors which might be considered as genuinely influencing cholesterol levels, the following conditions were observed:

1. The subjects were unmarried, young women on normal, regular diets, and were following a definite pattern of activity. Eleven of the subjects were nurses.

2. The determinations labeled as of the period of flow were obtained within several hours of the onset of bleeding, and again within several hours after the cessation of bleeding.

3. Although no absolute fasting period was maintained prior to obtaining each blood specimen for analysis, the same hour, and relationship to prior meal was observed.

4. Wherever altered routine was necessitated by night duty, this is so indicated in the tables. Such a period was used here as would not involve a change to day duty during the period of study.

The actual determinations were made by the author throughout, using the Schoenheimer-Sperry michrotechnique,[17] as modified for the colorimeter by Fitz,[18] and Shapiro et al.[19] The precautions against technical error were observed as outlined in a previous communication.[20]

Table 1 gives a complete outline of all determinations performed within this series. The complete range of total serum cholesterol values is from 118.2 mg./per cent to 249.1 mg./per cent. The free cholesterol values ranged from

33.4 mg./per cent to 77.5 mg./per cent. The percentage of free cholesterol ranged from 22.9 per cent to 32.9 per cent. All of the values are thus seen to fall within the range of values described by Sperry[13] and ourselves[20] (using the Schoenheimer-Sperry technique) as being found in "normal" series.

DISCUSSION

The most obvious characteristic of the data is the wide variation throughout the entire series. Certain features of this variability are remarkably constant however, such as the low total serum cholesterol at the onset of menses, and an elevated value at the termination of the flow. Associated with this is a rise in the percentage of free cholesterol at the onset of bleeding with a lowering of this percentage at the end of bleeding. This would indicate that it is the esterified cholesterol which is low at the beginning of the menses and rises rapidly during the bleeding phase. The 16 series of determinations in table 1, indicate these facts. It is useless, and pseudo-scientific to analyze thsee data with the usual statistical mensuration. There is no absolute rhythm of fluctuation, one may speak only of trends. This holds especially for the changes in blood cholesterol levels during the remainder of the cycle. Following the immediate post menstrual rise, the total cholesterol levels seem to be lowered and then to rise slowly until about the midpoint of the cycle, whence they fall to the low of the immediate pre-menstrual values.

The percentage of free cholesterol follows roughly this intermediate period of fluctuation. The fact that the percentage of free cholesterol at no time varies more than 10 per cent from lowest to highest values throughout the entire group, demonstrates the constancy of this measurement. A study such as this, wherein the total cholesterol values may vary so widely, yet where the percentage of free cholesterol remains relatively fixed, indicates the decreased sensitivity of this component to factors which change the total cholesterol levels. This increased stability must, therefore, give the fractionation of total blood cholesterol permanent importance as a clinico-chemical procedure. In addition, it may be seen that, although the fluctuations of the total blood cholesterol are quite wide for each individual

studied, the entire set of values per person apparently follow certain definite planes of variation. Discussion of this finding is reserved for another communication,[16] but it may be mentioned that the arrangement of such strata, is apparently along lines of constitutional types and behavior patterns.

To explain the pattern of blood cholesterol fluctuation which is observed in this study, there are several possible lines of physiological reasoning, none of which are subject to immediate proof in this relationship. An altered cholesterol:lecithin relationship may be involved in the breakdown of the uterine mucosal, vascular, infiltrated areas, which is the overt part of menstruation. Inasmuch as cholesterol is hemostatic, and lecithin is antagonistic to this, a lowering of the immediate premenstrual blood cholesterol by some endocrine effect, would aid in precipitating bleeding at the vulnerable point,—the uterine mucosa. Reversal of this mechanism would elevate the cholesterol level at the close of the bleeding phase—giving hemostatic action.

We personally feel that these cholesterol changes are an indication of another type of reaction in the female at the time of menses. As was conclusively shown by a previous paper on the rheumatic state,[11] the status of bodily resistance and reaction to disease bears a relationship, statistically, to the serum cholesterol levels. It is likewise an accepted fact that, just prior to the onset of the menses, many women with chronic disease states will suffer exacerbations, and many otherwise healthy females will have some associated pathological condition. That many body systems, chief among which is the reticulo-endothelial unit, are involved in alterations of the reaction to pathological processes, is well known. By reasoning through the blood cholesterol changes, it may be concluded that there are fluctuations in the status of the reticulo-endothelial system as a factor in response to disease, especially at the time of the menses.

SUMMARY

Data are presented for each of a total of 16 monthly cycles in 16 young, healthy and regular women. About 8 determinations

per cycle were performed. The following observations were made from this data:

1. There is a sharp elevation of the total serum cholesterol at the end of the bleeding phase, associated with a slightly lowered percentage of free cholesterol.

2. There is a lowered total serum cholesterol at the onset of the menses. The percentage of free cholesterol is higher here than at the termination of the flow.

3. The intermediary cholesterol levels show a tendency to rise to the middle of the cycle, and then fall off toward the new cycle.

4. Although there were wide fluctuations for each subject, no determination was outside of the values previously found in other series on normal subjects.

5. It is pointed out that there appear to be definite planes (i.e. higher, lower or intermediate levels) along which all of the total cholesterol values for any subject will vary. These planes are held to be determined, in part, by the constitutional-reaction type of the subject.

6. The narrow limits of fluctuation of the percentage of free cholesterol are pointed out as indicating the importance and stability of this factor. This variation is from 22.09 to 32.9 per cent of the total cholesterol.

7. The meaning of this variation of cholesterol values with regard to the menstrual cycle is discussed.

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HISTOLOGIC STUDY OF THE ENDOMETRIUM DURING PREGNANCY*

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Although the significance of chorionic villi and decidual cells is well known, little attention has been paid to the endometrial glands during pregnancy. Not infrequently, during the microscopic examination of an endometrium in which chorionic villi or decidua are not in evidence, the question of pregnancy is raised purely on the basis of certain changes that are manifested in the glands.

This investigation was undertaken to determine the reliability of such changes in connection with the question of pregnancy. Hence, a study was made of endometriums in cases of abortion, normal uterine pregnancy, extra-uterine pregnancy, and also in two cases in which examination of an agravid uterus revealed early loss of tissue.

As far as we have been able to ascertain, Leopold (1877) was the first to observe sinuous glands in the endometrium during pregnancy. Opitz (1899) described in detail the changes in the endometrial glands during pregnancy. He found that early in pregnancy the glands formed papillary processes which projected into the lumens and that the intervening stroma was scanty. The individual cells lining the glands were swollen and poorly stained. Later in the course of pregnancy, the papillary projections and the greater part of the epithelium disappeared and the glands were lined by one layer of cells. Opitz (1900) reported the results of a histologic study of the endometrium in 140 cases of abortion. He said that the glands were so typical

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in forty cases that a diagnosis could be made from them alone. He felt that the desquamation of the cells was a degenerative process secondary to abortion. Seitz (1903) said that pregnancy did not exist in one case in which the glands were considered typical of pregnancy according to the criteria used by Opitz. Opitz (1903) argued that the endometrial glands in the case described by Seitz were not typical of pregnancy because the interglandular stroma was too thick and the glands were not sufficiently numerous.

Teacher (1930) said that the earliest change in the development of the decidua was the formation of papilliform growths in the glands which became saw-toothed. He believed that the purpose of these glands was to nourish and aid the implantation of the ovum.

Boehmerus (1752) was the first to observe the formation of decidual tissue in the uterus in cases of ectopic pregnancy. Numerous writers (Schumann, 1921; Graves, 1928) have expressed the opinion that decidual tissue is formed in the uterus in every case of ectopic pregnancy, but that it is not uncommonly expelled as a cast at the time the tubal rupture occurs. Geist and Matus (1929) found uterine decidua in twenty-three of thirty-nine cases of extra-uterine pregnancy. Siddall and Jarvis (1937) studied the endometrium from thirty-eight cases of proved ectopic pregnancy. Decidual tissue was present in the uterus twenty-one times; it was present in all cases in which bleeding from the uterus had been present for less than eleven days. Moritz and Douglass (1928) were able to find decidual tissue in the endometrium in only eight of fifty-three cases of ectopic pregnancy. In twenty-nine of the remaining cases, the endometriums were in the so-called resting stage and in sixteen cases there was cystic hyperplasia of the endometrium. In a small proportion of the cases the authors were unable to obtain any history of uterine bleeding or expulsion of casts; they, therefore, concluded that decidual tissue was not formed in the uterus in all cases of ectopic pregnancy.

Knepper (1936) described a peculiar necrosis of the decidual

cells in the endometrium, which was present in the last half of uterine pregnancy but was constantly absent in the decidual cells of the endometrium in forty-seven cases of extra-uterine pregnancy. He did not say how frequently decidual tissue was formed in the uterus during extra-uterine pregnancy.

Hartje (1907) pointed out that glands similar to those found in pregnancy are found in the endometrium immediately preceding menstruation. Hitschmann and Adler (1908) proposed the term "decidual glands" for these glands and considered their presence as an indication of the premenstrual stage. O'Leary and Culbertson (1928) described decidua and tortuous saw-toothed glands which were present late in the menstrual cycle and which were similar to the glands of pregnancy which were described by Opitz. They believed that they were caused by an increased secretion of the glandular epithelium with the resultant dilatation and crowding of the glands.

Teacher (1930) expressed the opinion that the proliferation of the premenstrual phase corresponded to an early formation of decidua. He noted that all degrees of changes in the stromal cells, including definite decidual formation, could occur. Rock and Bartlett (1937) observed that after the twenty-fifth day of the menstrual cycle the stroma became edematous and the cells become large and had pale vesicular nuclei, which gave them the appearance of decidual cells. These changes he termed as the "predeciduum."

The findings in this paper are based on a study of the endometrium in 111 cases of intra-uterine pregnancy, twenty-seven cases of extra-uterine pregnancy (twenty-six cases of tubal pregnancy and one case of abdominal pregnancy), and two cases in which the uterus was agravid. In most of the cases of intra-uterine pregnancy the endometrium was examined early in the course of the pregnancy. The presence of chorionic villi was the criterion for the assumption of pregnancy in the first two groups of cases. In one case a biopsy revealed decidual tissue but no chorionic villi; the pregnancy continued and a full-term baby was delivered. No attempt was made to choose a selected

series of cases of uterine pregnancy; the cases included in this series are consecutive cases in which the existence of pregnancy was incontrovertible. In eighty-six cases of uterine pregnancy the endometrium was obtained by curettage either following incomplete abortion or in the course of therapeutic abortion, which was performed for obvious reasons. The most common indication for therapeutic abortion in these cases was pulmonary tuberculosis. In twenty-five cases of uterine pregnancy the uterus was available for study. In nine of these cases the pregnancy was an incidental finding at necropsy. In the remaining sixteen cases the uterus was removed surgically because of serious complications, such as multiple leiomyomas and rupture. In eighteen of the cases of extra-uterine pregnancy specimens of the endometrium were obtained by curettage, for diagnostic purposes, preliminary to exploratory laparotomy; in nine cases the uterus and the fallopian tube were removed because of multiple leiomyomas.

One piece, and occasionally several pieces, of endometrium from each patient was sectioned by the fixed frozen technic and stained with hematoxylin and eosin. Since the existence of pregnancy had been proved by the finding of chorionic villi, we selected only those sections in which the endometrial glands were evident. The results of the study of the endometrium were tabulated according to decidual formation, flattening of the endometrial glands, thinning of the interglandular connective tissue, papillary infoldings of the glands with serration or scalloping of the free border of the cells lining the glands, and degeneration of the cells lining the glands. Where it was possible the endometrium was compared with one of the phases of the menstrual cycle which have been described by Herrell and Broders (1935). They divided the normal menstrual cycle into four phases, each of which normally lasts seven days. These phases are the early and late proliferative phases and the early and late differentiative phases. The duration of the pregnancy was estimated either clinically or from the length of the fetus, if it were available.

RESULTS

Uterine pregnancy

In sixty-seven of the 111 cases of uterine pregnancy microscopic examination of the endometrium revealed a picture that was

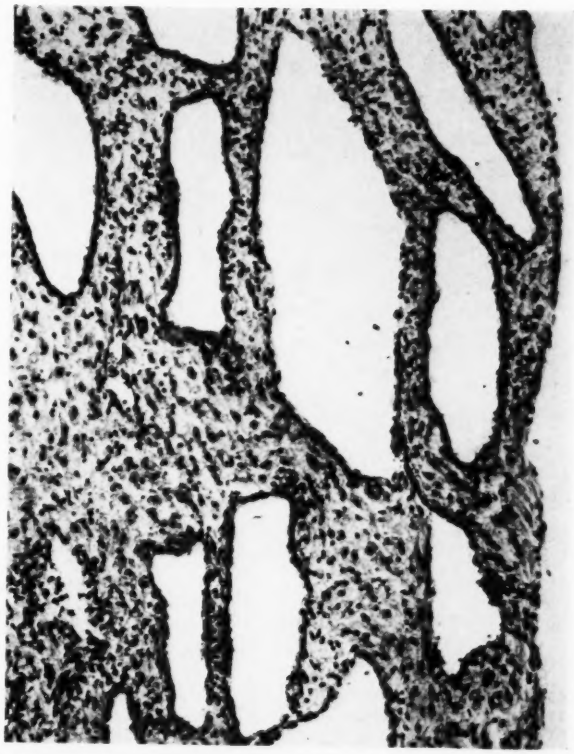

FIG. 1. Endometrium in a case of uterine pregnancy; the glands are surrounded by decidual tissue and the lining epithelium is flattened and one layer in thickness (hematoxylin and eosin × 90).

more or less comparable to the late differentiative phase of the menstrual cycle. The appearance of the glands varied a great deal depending upon the presence and amount of the decidual reaction. Where this decidual reaction was marked, the glands had lost their papillary infoldings, the glandular spaces were narrowed as if the decidua had encroached on them, and the lining

epithelium consisted of one layer which was flattened and in places was almost impossible to detect (fig. 1). In thirty-seven cases in which the foregoing change occurred it was impossible to compare the glands with those found during any part of the normal menstrual cycle. Many variations between the typical

FIG. 2. Endometrium in a case of uterine pregnancy; the glands show the transition from the typical "glands of pregnancy" to glands which are surrounded by decidual tissue and lined by a single layer of low cuboidal epithelium (hematoxylin and eosin × 100).

"glands of pregnancy" and this appearance were seen (fig. 2). In seven cases the endometriums differed in appearance from the endometriums in the group of sixty-seven cases and the group of thirty-seven cases. In three of the seven cases the endometrium was comparable to that of the late proliferative phase of

the menstrual cycle; in two cases there was evidence of a late proliferative phase and an early differentiative phase; in one case there was an early differentiative phase and in another case there was evidence of an early differentiative phase and late differentiative phase of the menstrual cycle.

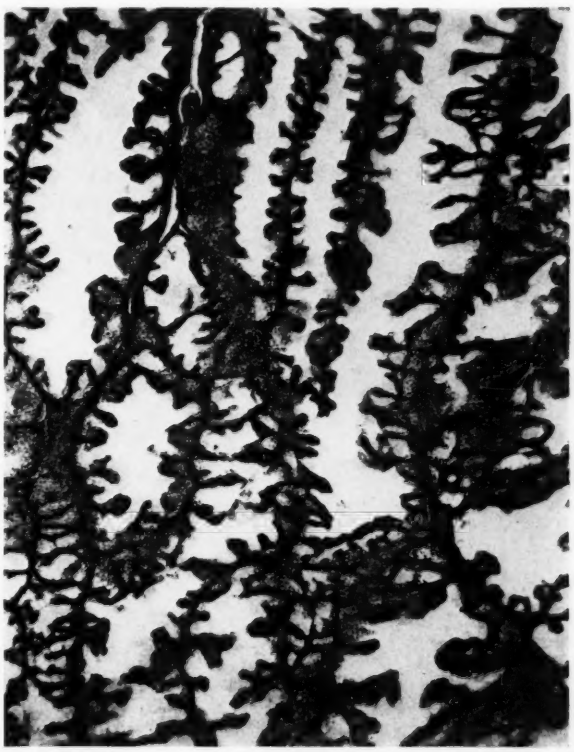

FIG. 3. Endometrium in a case of early uterine pregnancy; the glands show the typical saw-tooth appearance but very little interglandular stroma (hematoxylin and eosin × 50).

Decidual tissue was found in the endometrium in seventy-six cases. This varied from a slight swelling of the stromal cells to a marked formation of decidua.

The so-called glands of pregnancy were present in the endometrium in sixty-two of the 111 cases (fig. 3). They were evidenced either by their papillary infoldings or serration of the

free border of the glands, or both. The changes in the glands of the endometriums in these cases were manifested to such an extent that one could say with reasonable certainty that one was dealing with the "glands of pregnancy," irrespective of the presence of decidual tissue or chorionic villi. In thirty-six cases

FIG. 4. An endometrial gland in a case of uterine pregnancy; one may note marked degeneration of the lining epithelium with desquámation (hematoxylin and eosin × 210).

the glands were packed together so closely that there was little stroma or connective tissue between them. This was a characteristic finding, when it was present. The serration of the free border of the cells lining the lumen appeared to be caused by a peculiar degeneration of the epithelial cells characterized by

swollen and poorly stained cytoplasm. This resulted in small spaces between the superficial or free portion of the individual cells and bulbous projections of the cells into the lumens of the glands (fig. 4). The "glands of pregnancy" were found to be most typical in those cases in which decidual formation was

FIG. 5. Endometrium during uterine pregnancy, showing transitional stage of the glands, loss of papillary infoldings and a serration of the free border of the epithelial lining (hematoxylin and eosin × 205).

minimal; therefore, these glandular changes are most common in early pregnancy. As the decidual formation increased, the glands lost their papillary infoldings and serration (fig. 5), while the lumens of the glands became small. This ironing-out process appeared to be produced by an actual desquamation of the

degenerated epithelial cells (fig. 4). The final result was a regular small glandular space which was lined by flattened epithelium and surrounded by decidual tissue (fig. 1).

In eighteen cases the glands were flattened to such an extent that their longest diameters, instead of being perpendicular to the uterine cavity, were parallel to it. This could be explained on the basis of increased intra-uterine pressure caused by the products of conception.

A study of the basal glands was confined to those cases in which the uterus was available for examination. There were no noticeable variations from normal.

In twelve of the 111 cases it was not possible to presume pregnancy, either as a result of decidual formation or from the appearance of the glands. In two of these cases the patients were eight months and two months postpartum respectively, and menstruation had been resumed. There were two other cases in which prolonged metrorrhagia had followed an incomplete abortion; in these cases it seemed probable that ovulation had taken place. In seven of the twelve cases examination of the endometrium did not disclose a picture that was comparable to the late differentiative phase of the menstrual cycle, which was frequently associated with pregnancy.

Ectopic pregnancy

In the twenty-seven cases of ectopic pregnancy, when the changes in the endometrium were compared to the phases of the menstrual cycle the variation was much greater than that observed in the cases of uterine pregnancy. In ten cases the changes in the endometrium were more or less comparable to the late differentiative phase of the menstrual cycle; in 8 cases they were comparable to the late proliferative; in two cases they were comparable to the early differentiative phase and late proliferative phase, and in three cases they were comparable to the early differentiative phase. In four cases the glands were lined by a single layer of flattened epithelial cells and were completely surrounded by decidual tissue. In these cases the changes did not resemble any normal phase of the menstrual cycle. Decidual tissue was present in only five cases.

"Glands of pregnancy," in which there were papillary infoldings and serration, were present in seven cases (fig. 6). In seven other cases there was serration of the free border of the cells but no papillation; this change was not considered to be sufficient for a diagnosis of the "glands of pregnancy." Degeneration and a swelling of the epithelial cells were observed in five cases.

FIG. 6. Endometrial glands in a case of extra-uterine pregnancy; the typical papillary infoldings and bulbous projections of the cells may be seen (hematoxylin and eosin × 95).

There were markedly thin septa between the glands in two cases; in these cases the glands appeared to face one another. In one case cysts were found in the endometrium. In eleven cases the examination revealed typical decidua or glands of pregnancy, or both. In those cases in which the uterus was available for study, the basal glands did not vary from normal.

The endometrium of agravid women

Specimens of endometrium obtained from two agravid women were studied. The first patient had been passing membranous tissue from the vagina during each menstrual period. Micro-

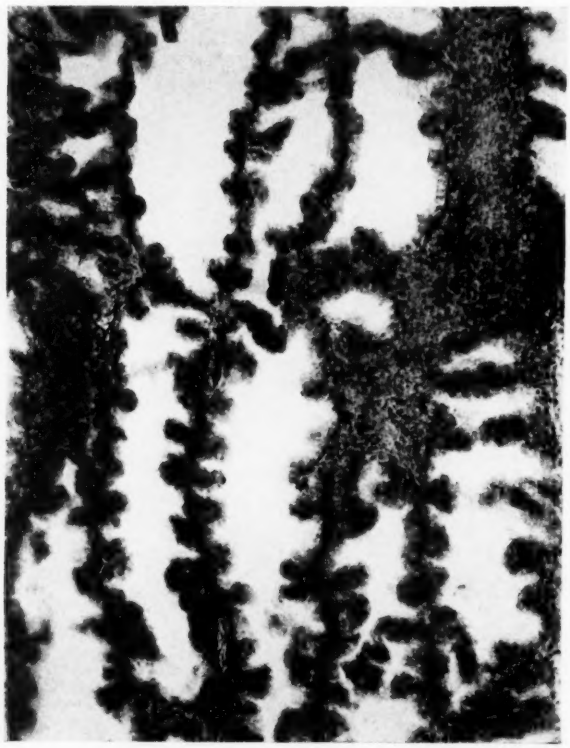

FIG. 7. Endometrium obtained from a nongravid uterus immediately before menstruation; the papillary infoldings and scanty interglandular stroma are shown (hematoxylin and eosin × 80).

scopic study revealed decidual tissue, small glands, and slight serration of the free border of the cells, but these changes were not sufficient to warrant a diagnosis of glands of pregnancy.

In the second case the endometrium was obtained by curettage, the day prior to the expected onset of menstruation. Examina-

tion of the endometrium revealed a very slight decidual reaction. The glands were in the late differentiative phase of the menstrual cycle, but there were papillary infoldings, serration of the free border, and degeneration of the cells, which are typical of the "glands of pregnancy" (fig. 7). In the endometrium there were collections of erythrocytes, which were indicative of early loss of tissue or beginning menstruation.

COMMENT

A picture more or less comparable to the late differentiative phase of the menstrual cycle was the most common finding in the endometrium in cases of uterine pregnancies. This picture was observed in more than half of the endometriums which were examined. These cases, together with those cases in which the glands were lined by a single layer of epithelium and surrounded by decidual tissue comprise approximately 95 per cent of the cases of intra-uterine pregnancy. The variations found in the endometrium in some of the remaining few cases of extra-uterine pregnancy can be explained on the basis of resumption of menstruation following incomplete abortion or delivery.

There was evidence of the late differentiative phase of the menstrual cycle in only ten of the cases of ectopic pregnancy. In the majority of the other cases there was evidence of the late proliferative phase. It is very difficult to explain this observation unless the menstrual periods continued uninterrupted.

The changes of the endometrial glands during pregnancy, as described by Opitz (1903), were marked in sixty-two of 111 cases of uterine pregnancy. These changes also were seen in seven of twenty-seven cases of ectopic pregnancy and in one case in which the uterus was agravid. The "glands of pregnancy" represent a variation of the late differentiative phase of the menstrual cycle. Most characteristic of this variation were papillary infoldings of the epithelium, swelling and degeneration of the cells and serration of the free border of the cells. In addition to the foregoing changes, the glands appeared to be more numerous than they are normally and the interglandular stroma was

very sparse. The apparent increase in glands is most marked early in pregnancy and disappears with the formation of decidual tissue. The epithelial cells were for the most part desquamated and the final result was a small glandular space lined by a single layer of cells with no papillary infoldings. It is evident that the glands have the same significance as does the presence of decidual tissue. They frequently are present in the early stages of a uterine pregnancy, in a small percentage of cases of ectopic pregnancy, and very rarely in cases in which the endometrium of an agravid uterus is examined immediately before menstruation. Their presence cannot be taken as absolute proof of an intra-uterine pregnancy.

Decidual tissue was present in only five out of twenty-seven cases of ectopic pregnancy. In six of the cases in which decidual tissue was not present, there was no history of vaginal bleeding or discharge. In only one case was there a history of the passage of a cast. These findings would agree with those of Moritz and Douglass (1928), who claimed that decidual tissue was probably never formed in the uterus in a number of cases of ectopic pregnancy. In a small number of cases in which the patients are normal, well-formed decidual tissue also is present immediately preceding each menstrual period.

In the majority of early uterine pregnancies in which chorionic villi are absent, one is able to make a correct presumption of pregnancy, even following abortion, with the aid of decidua and the changes in the endometrial glands.

CONCLUSIONS

1. A picture more or less comparable to the late differentiative phase of the menstrual cycle is usually present in the endometrium during the early part of uterine pregnancy; it is less common in extra-uterine pregnancy.

2. The changes in the endometrial glands ("glands of pregnancy") are present in a large proportion of cases of early uterine pregnancy, in a small proportion of cases of extra-uterine pregnancy, and occasionally in the agravid uterus, immediately prior to the menstrual period.

3. The "glands of pregnancy" have the same significance as decidual tissue.

4. Decidual tissue was found in the uterus in five of twenty-seven cases of extra-uterine pregnancy.

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PRIMARY CARCINOMA OF THE HEPATIC DUCTS*

Case Report

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Primary bile duct carcinoma or adenocarcinoma of the biliary canaliculi can no longer be considered a rare condition. However its occurrence is sufficiently unusual that statistics are still being compiled and presented in an effort to establish the average clinical picture presented by this disease. The necropsy incidence of primary bile duct carcinoma varies greatly in several reported series. In Strong and Pitts'[1] group of 1,967 consecutive necropsies there are 4 cases, an incidence of 1 in 492. Gustafson,[2] reporting recently on 24,400 cases, found an incidence of 1 in 1,162. McLaughlin[3] studied 9,523 cases with an incidence of 1 in 1,360, and Counsellor and McIndoe[4] had just one instance of this condition in the 5,976 cases which they studied. The increased incidence in the first series quoted appears to be due to the definitely higher racial susceptibility of many of the patients seen by those authors. While at least several hundred cases of this disease have appeared in the literature,[3] the importance of gallstones or of parasitic infestation as the chronic irritative etiological factor has not been determined. The questions of which symptoms predominate, the length of the interval between onset of symptoms and death, the association of cirrhosis of the liver with this condition, and the extent and location of metastases remain open questions.

The following is a report of a case seen recently at The George Washington University Hospital.

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P. L., a white male, married, 43, native of Hungary, entered the hospital September 18, 1937 complaining of epigastric pain, requiring codeine for relief, and exhibiting jaundice and emaciation. Symptoms had their onset five months before when the patient noticed a swelling in his upper abdomen. Three months later jaundice with pruritis, light colored stools and dark colored urine were noted, and the epigastric swelling was increasing. A fall, with injury to the right flank in the region of the liver at about this time, was accompanied by pain and an accentuation of the jaundice. The epigastric mass became constantly larger and about a month before admission codeine therapy was instituted for the relief of pain. During the two months preceding admission, the patient lost 32 pounds. He did not tolerate fatty foods. The past history was indefinite but suggested the possibility of scarlatina, diphtheria and typhoid fever. The right femur had been fractured. The family history revealed no specific evidence of malignancy although both parents and all ten brothers and sisters had died, many from unknown causes.

Physical examination revealed an emaciated, fairly well developed, deeply jaundiced white male, admitted with normal temperature, pulse and blood pressure. The conjunctivae were very icteric. The epitrochlear and inguinal lymph glands on the right side were palpable. A large hard irregular mass, not tender, was palpated in the epigastrium and the lower border of the liver was at the level of the umbilicus. The urine showed a few hyaline and finely granular casts and a very faint trace of albumin. The icterus index was 45.5 mgs. The blood count showed an average number of red and white blood corpuscles with a very slight predominance of lymphocytes and an average amount of hemoglobin. This situation persisted until the eighth week in the hospital when the number of red corpuscles had dropped to 2,910,000 with 60 per cent hemoglobin and 26,400 white cells, 78 per cent of which were segmented neutrophils.

The pain persisted steadily after admission and on the sixth day an exploratory laparotomy was performed during which the cystic portion of the liver was aspirated, about 600 ccs. of bile being obtained. The liver was found to be markedly enlarged, and, in addition to the cyst, contained many fairly firm nodules scattered on its surface. The adjacent viscera were firmly bound together. A small area of the nodular tissue was removed and on microscopic examination was believed to be a metastatic adenocarcinoma, grade 4 malignancy. Because of the anaplastic character of the predominating type of cell it was impossible to determine the origin of the primary tumor.

Subsequent treatment was essentially symptomatic. The temperature and pulse varied little from the normal after the immediate effect of the operation had passed. There was gradual aggravation of symptoms. Toward the end of the ninth week the patient began to expectorate small amounts of rust-colored sputum. The following evening he died.

In addition to the antemortem findings, autopsy showed the liver to weigh 2,800 gms. and to be involved in a mass of dense fibrous adhesions, and bound

to the diaphragm, stomach and transverse colon. Its capsule was smooth throughout a considerable portion of its surface and was slate grey in color. In the right lobe was a hard yellow mass about 15 cms. in diameter which was filled with thick yellowish fluid and necrotic material. Its internal surface was rough, irregular and indurated. Beneath the liver capsule in adjacent areas were found small, fairly firm tumor masses varying in size from 1 to 4 cms., irregular in outline. A few cystic nodules, containing from 5 to 15 ccs. of bile, were found. The lymph nodes adjacent to the biliary ducts were enlarged, indurated, uniform in consistency and appeared to be fibrotic. The cut surface of the liver was of a mottled dark brown color and bile stained. The gall bladder wall was thickened and covered by adhesions. One spherical stone of practically pure cholesterol, about 1 cm. in diameter was found in the cystic duct above its insertion into the common bile duct. In spite of the presence of a stone there was no obstruction to the flow of bile from the gall bladder to the intestine. The cystic duct did not show malignant change, but the hepatic duct was surrounded by hard enlarged lymph nodes and dense fibrous connective tissue. Microscopic examination of the tissues enclosing the hepatic ducts revealed the presence of numerous newly formed embryonic biliary canaliculi. These embryonic ducts were irregular in outline and composed of cells, many of which showed hyperchromatic nuclei and mitotic figures. Surrounding the hepatic ducts below the liver were extensive areas of dense fibrous connective tissue with invasion by undifferentiated immature cells. The liver tissue adjacent to the hapatic ducts showed no fibrosis. The stomach, intestines, pancreas and other abdominal organs showed no malignant invasion or other important pathological change. There was an area of infarction about 7 cm. in diameter in the lower lobe of the right lung. There was no evidence of malignant invasion in any part of the chest. Microscopic examination of the tissue from the nodules of the liver shows malignant invasion with ciliated columnar epithelial cells, arranged in typical acinar groups, and duct formation. Numerous cells, displaying mitotic figures are noted. Some areas of the liver show considerable necrosis and bile-stained tissue is seen in the central portion of the lobules.

COMMENT

Although cirrhosis of the liver has been found in the majority of necropsies of cases of primary bile duct carcinoma, it was not present in this case. Cholelithiasis is commonly found in this condition and was present in this instance. However, there was no malignant change adjacent to the stone or in any part of the cystic duct. It is difficult to believe that the stone could have been a predisposing factor, causing a chronic irritation of that part of the biliary tree which showed malignancy. The pain,

jaundice, and loss of weight commonly found, were outstanding features of this case. There is no conclusive evidence that the typhoid fever which the patient had earlier, had any physiological or morphological effect on the tissue which shows the malignant changes. The period from the onset of the first symptoms to death occupied about seven months. There is no way of showing what part, if any, the assoicated injury had in hastening the final fatal outcome. The metastases in this case were by direct extension to the liver and by way of the lymphatics to the regional lymph nodes.

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LEUKEMIA IN THE NEW BORN, WITH DEATH AT BIRTH FROM TRAUMATIC RUPTURE OF THE SPLEEN*

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Among the causes of death in the new born is the rare condition first described as Primary Anemia of the new born (Van Jaksch's Anemia), and now known and described under the general term of Erythroblastosis Fetalis.

Erythroblastosis is defined as "failure of maturation of myeloblasts in the bone marrow with consequent appearance in the blood of blast forms of red and white cells." The etiology is unknown, but it can be considered a fetal blood disease. Pehu, Trillat and Noel[6] suggest that "this condition is a fetal blood disease in that, although the expression is not identical, the cause lies in a profound disorder of hematopoiesis." They included congenital leukemia as erythroblastosis, "believing that the etiology was a disorder of blood morphogenesis." If tumors of the placenta can occur, as, for example, the writer last year reported[7] an angio-fibroma of the placenta, so likewise may tissue abnormalities like leukemia occur in the fetus.

The usual picture of erythroblastosis is as follows: The infant dies at birth cr shortly thereafter, the parents are normal, the labor is normal, and there are no birth injuries. There is slight jaundice, splenomegaly, and marked anaemia. The usual blood picture is: RBC average 1.8 million; HB averages 35 per cent; neutrophiles average about 47 per cent; myelocytes average about 3.6 per cent, with many nucleated reds and marked anisocytosis.

Pasachoff[1] cited a case (reported by Youland), in which the

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infant died five days after birth with slight jaundice and a spleen about three times the normal size. There was myeloid hyperplasia in the spleen, tonsils, and mesentery. The liver was not enlarged. Its blood showed RBC 1.75 million, HB 26 per cent, WBC 94400, neutrophiles 51 per cent, myelocytes 13 per cent. Youland[2] reported this case as "Primary Erythroblastosis failure with myeloid hyperplasia." Pasachoff in comment suggests that the picture was more like leukemia. In the light of this and other observations, frank leukemia in the new born should be distinguished from erythroblastic anaemia. According to Abt, there have been eight cases of leukemia reported, all under the age of two years, and these must have begun in utero. J. V. Cooke[5] reported in 1933 three cases of myeloblastic leukemia all under the age of one year. Grullee[4] says "when death occurs during the first few days of life, excluding brain hemorrhage, then death may be attributed to some splenic abnormality."

When there is a splenomegaly in the fetus, whether from malaria, septic swelling, hydrops fetalis, erythroblastosis, or leukemia, then from trauma in delivery rupture may occur, and some cases of ruptured spleen have already been reported.

CASE REPORT

Mrs. T., aged 22, was admitted to the maternal ward of the hospital. She had had four children—one living, one died of pneumonia, and two were still born (cause unknown). Her temperature was 97.6–99, except that on one day it went up to 102; the pulse was 66; respiration was 20; the Wassermann was negative; and the urine was negative. For last three weeks she had had some edema of her feet. The blood count was: WBC 17400 with mature neutrophiles 74 per cent, immature neutrophiles 10 per cent, lymphocytes 11 per cent, monocytes 4 per cent, and basophiles 1 per cent.

On April 26, 1933 she delivered herself of an eight months female infant whose heart was beating when delivered. The baby gasped twice and died, and an autopsy was made one hour later.

Autopsy report

A female infant fully developed and about 20 inches long. Its eyes were closed by the pressure of an improperly shaped head, the nose was retracted and flat, and the anus presented as a hole in the back in the lower sacral region.

The skin was somewhat jaundiced and cyanotic. The abdomen was extremely distended and filled with fluid. The circumference at the umbilical region was 44 cm. (18 inches), and on incision drained off about 300 cc. of clear bile-stained ascitic fluid. This was immediately followed by the flow of a large amount of dark fluid blood, evidently from a retroperitoneal hemorrhage, dammed back by the ascitic fluid. The thymus was 2 cm. across and 0.4 cm. thick and showed no gross abnormality. The lungs were apparently normal. The heart measured 3 cm. across and 4 cm. long; the foremen ovale was patent, measuring 5 mm. in diameter. The heart's blood was fluid and did not have the pale, thin appearance of anemic blood. The stomach and duodenum were normal in size, and were plastered to the lower surface of the liver by adhesions, with the upper side of the pylorus firmly adherent to the hepatic ligament. The left adrenal appeared normal while the right adrenal was slightly enlarged, being about the size of a hickory nut. The liver was enlarged and the cut surface was dark. It measured 16 by 11 by 8 cm. thick and weighed 200 grams (normal at birth 120–150). The gall bladder contained about 1 cc. of light colored bile. The spleen was greatly enlarged and mottled by numerous grayish white spots. It measured 11 by 7 by 5 cm. thick and weighed 125 grams (normal at birth 5–20). The lower end of the spleen was attached to the abdominal wall, bowels, and mesentery by adhesions and the lower edge presented a torn surface about 6 cm. across. The torn off stump was about 6 cm. across and 4 cm. long and was adherent to the small intestines. These torn surfaces were evidently the source of the abdominal hemorrhage. The pancreas appeared normal.

The brain appeared normal and showed no evidence of trauma or hemorrhage.

Specimens were taken of the thymus, liver, spleen, adrenal, and abdominal fluid; and smears were prepared from the heart's blood and the bone marrow.

Postmortem diagnosis. Apparent cause of death was hemorrhage, from rupture of the spleen in delivery. Other gross pathology—splenomegaly, an enlarged liver, abdominal adhesions and ascites.

Histological

Blood smears. There were many nucleated red and white cells, the ratio of nucleated cells of all kinds to normal red cells, being 224 to 1000, or 1 to 4. Assuming, from the appearance of the whole blood, that the total count of red cells would be approximately 2 to 4 million, then at this ratio there would be from one-half to one million nucleated cells of all kinds. Approximately one-half of these nucleated cells were nucleated red cells in various stages of mitosis, and the other half were leucocytes, i.e., from 250,000 to 500,000 of which 13 per cent were myeloblasts and 8 per cent were myelocytes. The following was the differential count: Granulocytes 35 per cent, of which there were 13 per cent myeloblasts, 8 per cent myelocytes, juveniles 6 per cent, mature neutro-

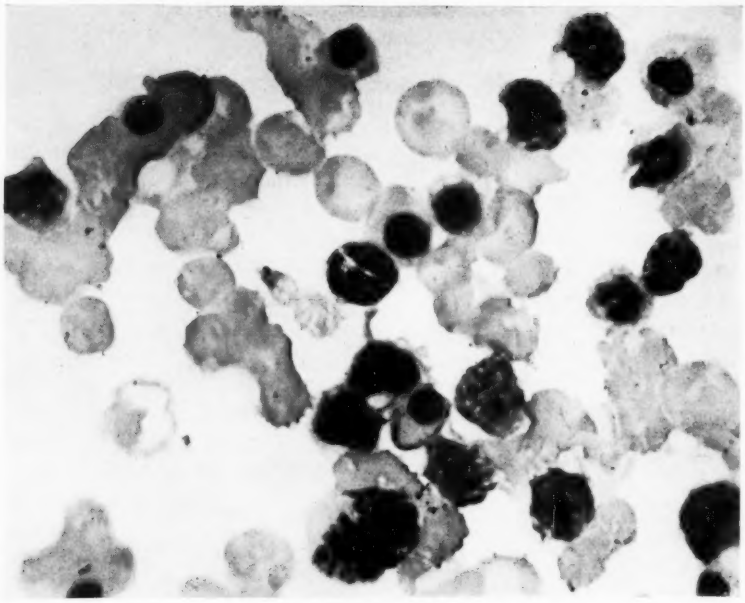

FIG. 1. SMEAR OF HEART'S BLOOD

Note proportion of nucleated cells (myeloblasts and transitional leucocytes, with mitotic figures) to normal erythrocytes. × 1000

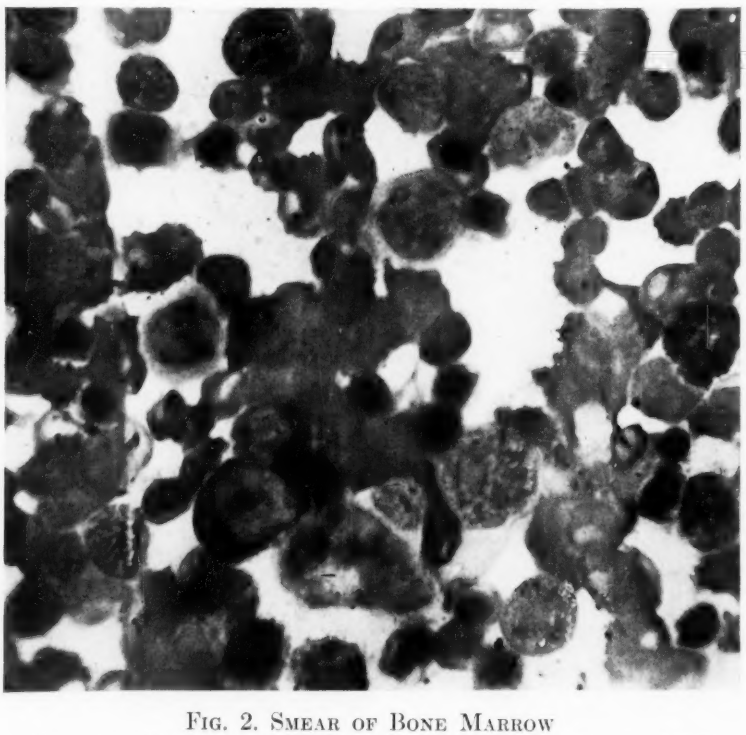

FIG. 2. SMEAR OF BONE MARROW

Large number of myeloblasts and nucleated red cells. × 1000

philes 7 per cent, and eosinophiles 1 per cent. There were 21 per cent transitional blast cells, 29 per cent lymphocytes, and 15 per cent monocytes. Many of the nucleated red cells showed active mitosis and there was much anisocytosis, poikilocytosis, and polychromophilia. Of the nucleated red cells per 100 cells, there were megaloblasts 37 per cent, normablasts 40 per cent, and microblasts 25 per cent.

Bone marrow smears. There were numerous myloblasts many showing mitosis, myelocytes, a few neutrophiles, and a few lymphocytes.

Abdominal fluid. Specific gravity 1012, color found due to bilirubin, blood cells present, bacteriological examination negative.

Cytology. Lymphocytes 90 per cent, plasma cells 5 per cent, and mesothelial cells 5 per cent.

The liver presented large lymph spaces and marked degeneration of liver cells. There was extensive myeloblastic and myeloid infiltration in the sinuses and reticulo-endothelial network.

The spleen was engorged with blood, the blood sinuses were large and the blood vessels dilated. The lymph follicles were broken down and largely replaced by an extensive diffuse infiltration of myeloid and myeloblastic cells. The trabeculae were thinned or absent due to edema and infiltration of myeloid cells and erythroblasts, thus accounting for its fragility.

The kidneys showed marked congestion, and granular degeneration of the tubular cells. The interstitial tissue was infiltrated by large myeloid cells.

The thymus and adrenal both showed myeloid infiltration. The adrenal was engorged with blood.

Final diagnosis. The pathology corresponds with that found in myelogenous leukemia, and a splenomegaly; and the cause of death was rupture of the spleen at delivery.

(The blood smears were submitted to Dr. F. J. Heck of Rochester, Minn., and to Dr. N. Rosenthal of New York City. The sections were submitted to Dr. A. C. Broders of Rochester, Minn.)

Comment. "I think this case is probably best classified as an erythroblastosis, although there are definitely immature myeloid cells present. I see no good reason why it could not equally well be called a case of leukemia in which the predominating stimulation has been on the nucleated red cells."—Dr. Heck.

"This is a very unique and unusual case. The blood smear shows a tremendous increase in the number of nucleated erythrocytes in all stages of development, and there are also immature leucocytes. The case is one of erythroblastosis fetalis, and of all the cases that have come to my attention, I have not met with the complication of a ruptured spleen in the new born."—Dr. Rosenthal.

"I agree that this is a case of myelogenous leukemia."—Dr. Broders.

Among other pathologists, Drs. R. R. Kracke, F. M. Johns and A. A. Foord agreed that this case should be classified as a leukemia.

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PULMONARY SCLEROSIS*

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According to some observers, sclerosis of the pulmonary arteries is quite common and this is probably true, if one includes all degrees down to the small occasional atheroma which one sees as part of a general systemic sclerosis in individuals past the fifth decade. Brenner[1] states he found evidence of sclerotic changes in some part of the pulmonary vascular bed in 97 per cent. of an unselected consecutive series of autopsies. Other observers note a smaller incidence ranging from 6.5 per cent. in 770 cases by Moschowitz[2] and Steinberg's[3] 65 per cent. in 500 unselected autopsies. These variations probably result from the differences in basic criteria as to what should constitute a significant sclerosis. However, the cases in which the degree of sclerosis is so severe as to be the predominating feature and to far overshadow the systemic sclerosis are, indeed, uncommon. The following is a case which would fall in this group.

REPORT OF CASE

History. The patient, a colored male of uncertain age, variously stated from 39 to 50, was admitted to the hospital complaining of dyspnoea, cough, vomiting and diarrhoea. He had worked as a chauffeur until about eighteen months prior to admission when he changed occupations to become a caretaker of a small estate. He worked at this for six months and one day suddenly collapsed, the cause of which he attributed to overwork. He was forced to go to bed for several days to rest. Following this interlude he returned to work but was unable to carry out his duties as he tired too easily and became short of breath upon the slightest exertion. This condition persisted and finally, a few months ago, he developed a cough which was productive of a whitish, tenacious sputum. These symptoms became more aggravated and about three days

* Received for publication March 25th, 1938.

ago he became acutely ill with pain in his abdomen which was followed shortly by nausea and vomiting. A diarrhoea then developed and the pain was somewhat relieved. Following this attack he was seized with a chill, which lasted from two to three hours, and was then admitted to the hospital. He stated he had had no loss of weight or pains in his chest but that on various occasions he had had night sweats.

Past history. Measles, mumps, whooping cough and pneumonia.

Family history. Essentially negative.

Physical examination revealed an emaciated colored male appearing to be in the fifth decade of life, weighing 120 pounds and approximately six feet tall. The finger tips and mucous membranes showed marked cyanosis. There was no cutaneous eruptions or edema. There was bilateral diminished expansion with impaired resonance and many crepitant and moist râles over both lung fields. The heart was enlarged to the right. The apex beat was within normal limits. There was a suggestion of gallop rhythm. A systolic murmur was heard at the base and apex. The pulse rate was 110; the blood pressure was 125 systolic and 90 diastolic.

Roentgen examination of the chest showed extensive mottling throughout both lung fields with exception of apices which were clear. This mottling was more pronounced in the middle thirds. The heart showed marked enlargement in its transverse diameter with considerable widening of the aorta.

Electrocardiographic tracings on two occasions revealed delayed A–V conduction, right axis deviation, sinus tachycardia and myocardial damage.

Urine negative, Kahn 4+, and sputum examinations on numerous occasions revealed no tubercle bacilli.

Examination of the blood showed 5,020,000 red blood cells; 10,800 white blood cells; 69 per cent polymorphonuclears, 5 per cent band forms, 1 per cent eosinophils, 18 per cent lymphocytes and 7 per cent monocytes.

The patient was treated symptomatically, and with bed rest, but respirations gradually became more labored and he expired on the twenty-second hospital day.

Necropsy. The body was that of an emaciated, colored male, apparently about 55 years of age. The lips and gums showed marked cyanosis and congestion with a definite blue line at the base of the teeth. The nail beds were also cyanotic. Upon opening the thorax and abdomen, the organs occupied their normal positions.

The left pleural cavity was obliterated by dense fibrous adhesions. The lung weighed 635 grams, was subcrepitant throughout and somewhat nodular in consistency with numerous emphysematous blebs along the periphery, more marked at the apex. On section (fig. 1) the cut surfaces were markedly congested and presented evidence of marked fibrosis with an apparent increase in number and size of bronchioles. The pulmonary arteries stood out prominently, showing marked thickening by numerous atheromatous plaques. Near

the hilus the fibrosis appeared denser and an occasional small, yellowish nodule suggesting a healed tubercle was noted. The right lung was similar to the left and weighed 650 grams (fig. 1). The consistency was also nodular and, on section, presented much the same picture as the left except there was no gross evidence of tuberculosis.

The heart was enlarged and weighed 475 grams, with the apex formed by the dilated right ventricle. The right auricle was markedly dilated and the wall hypertrophied, in some places up to 4 mm. in thickness. The tricuspid valve leaflets showed mild thickening and fibrosis at the periphery but no specific inflammatory changes.

FIG. 1. CROSS SECTION OF LEFT AND RIGHT LUNGS RESPECTIVELY, SHOWING THE MARKED SCLEROSIS OF THE BRONCHUS OF THE PULMONARY ARTERIES

The myocardium of the right ventricle was markedly thickened, measuring from 9–10 mm. in thickness, with great hypertrophy of the columnae carneae and papillary muscles. The pulmonary valve leaflets were thickened and fibrotic, two being fused into one with an imperfect division between. There was also sagging at the commissures. The pulmonary artery appeared somewhat dilated and showed several atheromatous plaques measuring up to 2.5 cm. in diameter.

The left side of the heart was not remarkable, showing only a mild dilation of the left ventricle. The wall measured 15 mm. in thickness. The coronary

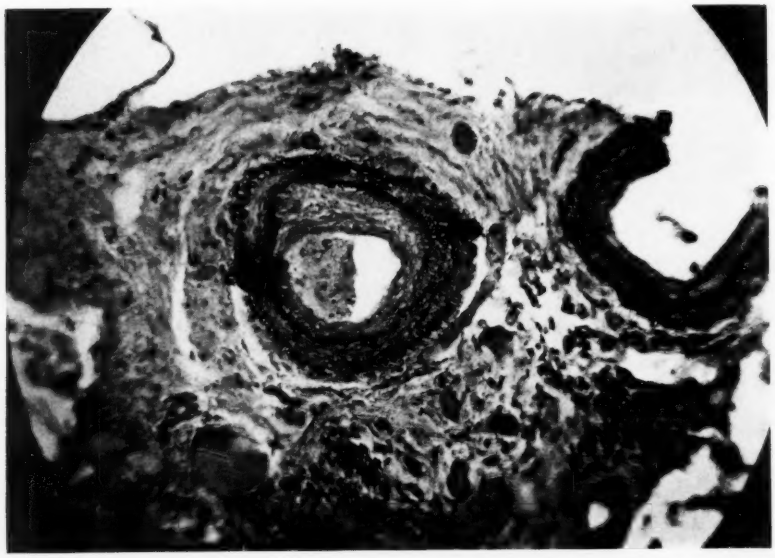

Fig. 2. Microphotograph of One of the Smaller Arteries Showing Marked Intimal Thickening (× 150); Verhoeff's Elastic Tissue Stain

Fig. 3. Microphotograph of Larger Artery (× 150); Verhoeff's Elastic Tissue Stain, Showing Marked Eccentric Intimal Thickening and Fragmentation of Internal Elastica Lamina

arteries showed a rather marked degree of sclerosis but there was no definite obstruction. The aorta, likewise, showed a marked sclerosis.

The gastrointestinal tract was essentially negative, presenting no evidences of ulcerations, obstructions, or neoplasms.

The spleen weighed 90 grams and showed marked congestion.

The pancreas showed mild fibrosis and congestion.

The kidneys showed moderate congestion with mild toxic changes but no specific lesions. The adrenals showed only a mild congestion.

The bladder and prostate were negative.

The liver weighed 1,350 grams and, on section, revealed the mottled appearance of chronic passive congestion. The gall bladder contained no stones.

Anatomical diagnosis. Pulmonary arteriosclerosis associated with bilateral pulmonary fibrosis: bilateral bronchiectasis associated with emphysema. Cardiac hypertrophy and dilatation of the right heart. Generalized arteriosclerosis. Chronic passive congestion of the liver, spleen, and kidneys.

Microscopic sections from blocks fixed in formaldehyde and stained with hematoxylin and eosin revealed marked fibrosis in the lungs with congestion of the blood vessels and capillaries. There were areas of extravasation of blood into many alveoli. The pulmonary arteries and arterioles showed marked intimal thickening which was quite evident in the sections stained with Verhoeff's elastic tissue stain (figs. 2 and 3). Occasional arteries appeared almost obliterated. There was no evidence of tubercles noted in any of the sections. There was no perivascular cuffing, nor was there any sign of spirochaetes noted in any of the sections stained with Levaditi's method. The bronchioles were dilated and the peribronchial tissues were infiltrated with numerous lymphocytes with occasional monocytes. Many large pigmented mononuclear phagocytes were scattered throughout the section, mostly in the alveoli.

Sections of the heart showed a few small areas of fibrosis and hypertrophy of the myocardial fibers.

The pulmonary artery and aorta showed no specific perivascular round cell infiltration suggestive of lues.

Sections of the remaining organs were not remarkable.

COMMENT

The cases of so-called Ayerza's disease are probably related to this group but on careful examination of those cases listed as such, the conclusion is reached that Ayerza's disease is not a distinct entity but merely a clinical syndrome and that any number of factors other than pulmonary sclerosis may be responsible for the symptoms. Generally it has been classified as a condition in which the patient has heart failure with intense cyanosis and, at autopsy, is found to have syphilis of the pulmonary arter-

ies and, possibly also, of the lungs and bronchi. However, Warthin[4] in 1917 described a case of syphilis of the pulmonary arteries and demonstrated Spirochaeta pallida in the lesions but the patient had no such symptoms as described by Ayerza, adding further proof that the so-called Ayerza's disease is a syndrome and not a distinct pathological entity, especially so, since Ayerza described, in an unpublished clinical lecture, a case with heart failure presenting such marked cyanosis as to be almost black (Cardiaco negro), and failed to mention any lesions of the pulmonary arteries. The principal findings at autopsy were dilatation of the bronchi, peribronchitis and hypertrophy and dilatation of the right auricle and ventricle, with the primary pathology located in this region. It would hardly be likely that he overlooked lesions of the pulmonary artery, had they been present, especially to the degree of being of significance in the production of the symptoms.

The etiologic factors in pulmonary sclerosis are, in all probability, basically similar to those in systemic sclerosis and likewise can not, in most cases, be blamed on any one factor but are a combination of stress, strain and irritations, mechanical, toxic or otherwise. Rosenthal's[6] cases appeared to be associated with constant aspiration of particulate matter (sand, iron), or fumes, (naphtha, benzene), which he believed led to spasm and necrosis of capillary endothelium. Bacon and Apfelbach[7] regarded influenza as playing an important etiological rôle, Sokoloff and Stewart[8] suggested that their case resulted from a combination of endocrine and neurologic factors and many others including Rogers[9], Warthin[4], Hare and Ross[10], Bruce and co-workers[11] and Cheney[12] believed syphilis to be the primary cause.

CONCLUSION

Although the Kahn reaction was 4+ in this present case there is no microscopic evidence such as the presence of spirochaetes in the lesion, or the presence of perivascular round cell infiltration to prove that syphilis played any part in the production of the sclerosis. The gross evidence of emphysema, chronic bron-

chitis and bronchiectasis would seem to be the primary etiological factors.

It is further concluded that this type of lesion should not be referred to as Ayerza's disease.

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EDITORIAL

On Chemical Specificity In Growth And Development

Chemical specificity is a part of the big problem of the chemical and physical bases of morphogenesis, as well as of other problems of cell activity. How can we correlate morphogenetic factors, i.e., those processes which cause organisms to grow, develop and maintain definite structures, with chemical compounds, groups, molecules and atoms, and with physical activities?

An immediate second question is—what changes occur when morphogenetic factors are disturbed or completely eliminated as they are in malignancy?

The working hypothesis that the cell is an ordinary colloidal mechanism no longer is sufficient to account for the facts. Recent work has detected great importance in smaller molecules; in fact, not necessarily in molecules, but in various chemical groups within molecules.

Coupled with the ideas and the demonstrations that not only are the chemical molecules themselves with their various groups and atoms of importance in morphogenesis, is the amply justified conception that the positions in space and the shapes of the molecules determine many morphological peculiarities. As an instance, certain large protein molecules are not necessarily globular, but many may be fibrillar or practically two dimensional. Furthermore, studies of surface layers have shown that certain molecules stand up with one end dipping into one medium and the other end projecting into the other, like the bristles in a brush.

A useful working hypothesis is that which regards the molecules of smaller dimension as producing a sort of skeleton within cells, in the meshes of which are the larger molecules constituting a colloidal matrix. With the innumerable and almost unbelievably large surfaces exposed by such arrangements, there is opportunity for not only enormous varieties of reactions and

interreactions, but also for great speed in them; in addition, it allows of the working hypothesis of how there can be a sort of stability in the midst of lability, that is, a more or less permanent architecture in which those changes are constantly taking place which have been called the "dynamic equilibrium of life."

But the immediate subject is chemical specificity, which is, as stated, part of the problem of morphogenesis.

Putting the question in this form is perhaps an easier way of approach. Chemists tell us that within cells are many compounds and then proceed to name large numbers of them. As students of growth we ask the question—what do they do in there in relation to growth and development? Does each have its specific part to play in these processes, and if so, what? Conversely, if growth is disturbed, is it because of lack of these compounds or because they cannot be properly integrated into the teeming chemical activities of the whole milieu?

Possible methods for studying the function of intracellular compounds may be those of subtraction by removing certain compounds from within cells, or addition, by adding them. This extremely difficult field, involving many and the utmost hazards is but in its infancy. Another method by which the same addition and subtraction ideas may be carried out, is by depriving the cell of certain substances in the environment from which it abstracts its materials, or by adding materials in larger amounts than ordinarily occur in the normal environment of the cell.

The best type of organism for such studies would seem to be one which lives a comparatively short life cycle, which is transparent, or at least translucent, which can be observed under the microscope for hours or days and which develops by a series of separable growth and developmental phases. If these latter can be correlated with anabolism, maintenance metabolism and catabolism, so much the better.

Recent work makes it possible to give a direct answer to the original question. Many normally occurring intra-cellular compounds play specific parts in one or the other of the phases of growth and development. As instances, the sulfhydryl group and its derivatives are active in cell multiplication.[1] d-Glutamic

acid, l-aspartic acid, l-proline and l-hydroxyproline enhance the differentiation of cells, as do tyrosine, cytosine and thymine. It would seem as though there might be some common relationship between these compounds corresponding to this common functional activity. Careful examination of the chemical constitution and reaction possibilities shows that there is through the pyrrolidone configuration or some part or derivative of it.[2]

STANLEY P. REIMANN

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NEWS AND NOTICES

Convention News

Another Convention has come and gone and, true to tradition, proved more than the equal of those of the past. Distance proved no obstacle as was shown by a registration of 108 members and 54 visitors.

Though the scientific exhibits were, perhaps, not as numerous as in other years, their lack in numbers was made up by their general excellence. The gold medal award was made to Doctor Benjamin T. Terry for his exhibit "Aids In The Rapid Diagnosis of Tissues."

The commercial exhibits, as usual, were instructive and worth while.

The Scientific Session was of particular interest as will be seen in the papers to be published.

Like its predecessors, the Tumor Seminar was of outstanding interest and value. It was very well attended, having a registration of 133 of whom 75 were members of the A. S. C. P.

The banquet was a colorful affair, enlivened by the presence of many ladies. The speaker of the evening was Dr. William Dock who presented an interesting review of the outstanding theories on pernicious anemia. His address will later be published.

At the Business Session many matters of importance were discussed, an account of which will be sent to each member through the Secretary's office.

At the Annual Election the following officers were chosen to serve with Dr. Thomas B. Magath, the incoming President:

President-Elect: Dr. L. W. Larson.
Vice-President: Dr. W. Cummins.
Executive Committee: Dr. C. W. Maynard, Dr. O. W. Lohr.
Board of Censors: Dr. W. S. Thomas, Dr. I. A. Nelson.
Board of Registry of Technicians: Dr. P. Hillkowitz, Dr. K. Ikeda.

A list of those elected to membership will be found below.

New Members to Whom We Extend Welcome

Alburger, Henry R.....Indianapolis, Indiana
Andujar, John Jose.....Little Rock, Ark.
Backus, Glenn R.....Flint, Michigan
Baker, Charles Preston.....Omaha, Neb.
Bayley, Wm. E. G.....LaCrosse, Wis.
Beam, Mark P.....Albuquerque, N. Mex.
Beliveau, Romeo A.....Lewiston, Maine

Benjamin, E. L. ..... Evanston, Ill.
Brilmyer, George J. ..... Washington, D. C.
Budd, John Wesley ..... Los Angeles, Cal.
Carr, Jesse, L. ..... San Francisco, Cal.
Derby, Irving M. ..... Brooklyn, N. Y.
DeVeer, J. Arnold ..... Ozone Park, N. Y.
Diggs, L. W. ..... Memphis, Tenn.
Dodds, Wemple ..... Crawfordsville, Ind.
Fenton, C. C. ..... Morgantown, W. Va.
Guttman, Paul H. ..... Sacramento, Cal.
Hala, William W. ..... Long Island City, N. Y.
Hertzog, Ambrose J. ..... Minneapolis, Minn.
Hobbs, R. E. ..... Shenandoah, Pa.
Hospers, Cornelius A. ..... Chicago, Illinois
Humphrey, A. A. ..... Battle Creek, Mich.
Jones, Maurice L. ..... Wichita, Kansas
Kastler, Franz ..... Rutherford, N. J.
Kilman, Joseph Erle ..... Wingdale, N. Y.
Kushner, Alexander ..... Rahway, N. J.
Leary, Olga Cushing ..... Boston, Mass.
Lebowich, Joseph ..... Saratoga Springs, N. Y.
Lennon, H. C. ..... Philadelphia, Pa.
Levine, Victor ..... Chicago, Illinois
Love, John W. P. ..... Johnson City, N. Y.
MacNeal, Ward J. ..... New York, N. Y.
Mayhew, J. Morgan ..... Greenburg, Pa.
McNaught, James Bernard ..... San Francisco, Cal.
Mermod, Camille ..... Oakland, Cal.
Meyer, Leo M. ..... Brooklyn, N. Y.
Miller, Ralph English ..... Hanover, N. H.
Mills, Harlan P. ..... Phoenix, Ariz.
Moss, Emma Sadler ..... New Orleans, La.
Mueller, Emil T. ..... North Tonawanda, N. Y.
Parkhill, Edith M. ..... Rochester, Minn.
Patterson, James Nelson ..... Jacksonville, Fla.
Payne, C. Allen ..... Detroit, Michigan
Penke, Madeline ..... Corona, N. Y.
Portuondo, B. C. ..... St. Louis, Mo.
Queen, Frank B. ..... Denver, Colo.
Roberts, John Richard ..... St. Louis, Mo.
Robertson, H. E. ..... Rochester, Minn.
Rogers, Helen Bush ..... Louisville, Ky.
Rohdenburg, George L. ..... New York, New York

Saccone, Andrea.....New York, N. Y.
Schultz, E. W.....Stanford University, Cal.
Silliphant, William M.....Pearl Harbor, T. H.
Straus, Reuben.....Cleveland, Ohio
Strong, Kenneth.....Brooklyn, N. Y.
Thurston, Eric W.....Chicago, Ill.
Walters, Albert R.....Sherbrooke, Quebec, Canada
Weiss, Charles.....San Francisco, Cal.
Wiener, Alexander S.....Brooklyn, N. Y.
Wollenweber, H. L.....Baltimore, Maryland

Elected to Associate Membership, 1938

Englander, Charles.....Newark, N. J.
Faught, F. A.....Philadelphia, Pa.
Hess, Charles L.....Bay City, Michigan
McLeod, K. W. A.....Detroit, Michigan

Honorary Members

By unanimous vote of the Society honorary membership was conferred upon Dr. Frederic E. sondern of New York and Dr. Manuel Martinez Baez of Mexico City.

That this is a well deserved honor is evident from the brief notes appended.

Dr. Frederic E. Sondern

Dr. Frederic E. Sondern was born on the 30th of March, 1867, in Stuttgart, Germany, while his parents, naturalized Americans, were traveling abroad. His early education was obtained in the public schools of New York City, where he graduated from high school at the age of sixteen. He then spent the next three years at the Universities of Heidelberg and Tübingen. Returning to the United States in 1886 he enrolled in the College of Physicians and Surgeons of Columbia University, graduating therefrom in 1889. He next became a member of the interne staff of Lenox Hill Hospital. Here he came under the eyes of Dr. Abraham Jacobi, with whom he became associated for the following six years. During these years Dr. Sondern gained enormous clinical experience both in Dr. Jacobi's extensive private practice as well as in various hospitals with which he became connected. At about this time, clinical pathology was forging ahead, soon to become a special field in clinical medicine, and Dr. Sondern's deep interest in hematology, bacteriology and biochemistry caused him, in 1898, to establish the first private clinical laboratory in New York City and henceforth he devoted his time exclusively to this specialty. Dr. Sondern became Professor of Clinical Pathology at the New York Post Graduate Medical School and Hospital, Director of the Clinical Laboratories of the New York Lying-In Hospital, Clinical Pathologist at Roosevelt Hospital and to the

Laboratory of the Out Patient Department at Bellevue Hospital and thus did much to establish and elevate the specialty of Clinical Pathology. In addition, Dr. Sondern has held many positions of honor and trust. He has for years been a delegate to the American Medical Association. He has served on practically every committee of the New York County and New York State Medical Society, was President of the County Society in 1916 and the State Society in 1935. As Treasurer he did much in placing the finances of the State Society on a sound and equitable basis, and gave the New York State Medical Society a bi-monthly Medical Journal which is a model of its kind.

For many years Dr. Sondern was Secretary and later Chairman of the Public Health Committee of the New York Academy of Medicine and has been a Trustee of this institution for a decade or more. He is a Past President of the American Society of Clinical Pathologists, a member of the International Society of Urologists, American Society of Pathologists and Bacteriologists, American Society of Immunologists, New York Pathological Society, New York Clinical Society, German Medical Society of the City of New York, and for many years, served as President of the Board of Trustees of the New York Post Graduate Medical School and Hospital and Trustee of the Good Samaritan Dispensary.

His publications in his specialty are numerous and his many contributions to laboratory medicine, especially on leucocytosis, etc., are too well known to require further elaboration.

Dr. Sondern's genial personality, his intimate knowledge, not only of his chosen specialty, but of the many problems confronting the profession as a whole, and his sympathy for the younger man in medicine, have endeared him to all who have had the privilege of coming in contact with him.

Dr. Manuel Martinez Baez

Dr. Manuel Martinez Baez was born in Mexico and has been one of the most important Pathologists in Mexico for many years. He is Dean of the Biological Division of the new Polytechnical School in Mexico City and Professor of Pathology at the Medical School.

He has a number of official duties, among which is the installation and supervision of safe water supplies for municipalities throughout Mexico. In addition, he is technical supervisor of the sanitary condition of food in Mexico, D. F.

He has studied in Europe and was for several years connected with the Pasteur Institute in Paris. He has published extensively on the pathology of Hodgkin's Disease, with particular reference to visceral lesions. He is a well-known authority on onchocercosis and has in recent years carried on extensive investigation on the pathology of mal de pinto. His studies on pathology of the skin are well known.

The important official position that Manuel Martinez Baez holds in Mexico and his thorough training as a Pathologist, put him in a commanding position in the medical profession in Mexico; and his interest in elevating the standards

of Pathology in his native country give him a very real common ground with members of the American Society of Clinical Pathologists.

At a recent meeting of the New York State Society of Pathologists, a constitution was adopted and the following officers elected:

President: Dr. W. S. Thomas.

Vice President: Dr. A. V. St. George.

Secretary-Treasurer: Dr. M. J. Fein.

Counsellors: Dr. S. H. Curtis, M. E. Marten, S. Weintraub, Herbert Brown, and Wm. A. Wall.

Committees were appointed for the specific purpose of undertaking to improve the economic situation of pathologists in the State of New York.

The 67th Annual Meeting of the American Public Health Association will be held in Kansas City, Mo., October 25–28, comprising fifty morning and afternoon meetings arranged by the ten Sections of the Association which are: Health Officers, Laboratory, Vital Statistics, Public Health Engineering, Industrial Hygiene, Food and Nutrition, Child Hygiene, Public Health Education, Public Health Nursing, Epidemiology.

There will be symposia on industrial hygiene administration, venereal disease control, laboratory diagnostic methods, expanding responsibilities in public health engineering, maternal and child health, frozen desserts, industrial hazards, water and sewage, typhoid fever, the next steps in school health services, milk and dairy products and many other important subjects.

Assembly of Laboratory Directors and Serologists, Hot Springs National Park, Arkansas, October 21–22, 1938

The intensive campaign to stop the spread of syphilis now being waged throughout the country makes it imperative that only those serologic tests of proved efficiency be made available to private physicians and health officers. Diagnosis of syphilis must be prompt and accurate. The serologic blood test, becoming positive within two or three weeks after the onset of primary syphilis and remaining positive in the vast majority of untreated patients throughout the entire course of the disease, is the most important evidence of the existence of syphilis.

The American Society of Clinical Pathologists in cooperation with the U. S. Public Health Service realized the need for reliable serodiagnostic tests several years ago. The work of the Committee on Evaluation of Serodiagnostic Tests for Syphilis is sufficiently well known to require no comment. It is the opinion of this Committee that its studies of the efficiency of the performance of serologic tests have progressed to a point where material gains would be made by a thorough discussion on common ground in which all those interested in the control of syphilis through laboratory methods may participate.

Plans are being developed for an assembly of laboratory workers from the

entire country. All such workers both from private, hospital and public health laboratories, as well as physicians and health officers interested in the control of syphilis, are invited to attend.

The proposed meeting, under the auspices of the Committee on Evaluation of Serodiagnostic Tests for Syphilis, with Surgeon General Thomas Parran, Chairman, is scheduled for October 21st and 22nd, 1937, at Hot Springs National Park, Arkansas.

The aims and purposes of the assembly will be to consider means and methods to improve and to make more generally available the serologic tests, which are so important in syphilis control work. Tentative arrangements call for the presentation of the program in four sections.

The first section will consider the need for adherence to conventional technic in the routine performance of reliable serodiagnostic tests. This subject will be considered in papers by Doctors Harry Eagle, William A. Hinton, Reuben Kahn, Benjamin Kline, and John A. Kolmer, with special reference to the tests which each of these workers has described.

Need for training of laboratory personnel will be the subject of the second section. The qualifications and training for both laboratory directors and technicians will be presented in separate papers.

The third section will discuss the prosecution of the studies to evaluate the performance of serologic tests within the States The efficiency of branch State laboratories and of municipal, hospital and private laboratories cannot be studied on a national basis. The subject is much too large. Should this be made a function of the State or large municipal department of health? Actual experience with such studies in the States of Maryland and New Jersey and in the City of Cleveland will be described.

The fourth section will consider the desireability of licensing or approving for the performance of serodiagnostic tests for syphilis, laboratories within the States by the respective State departments of health. This discussion will be conducted from the standpoint of the private laboratory director by Dr. Frederick H. Lamb of Davenport, Iowa. The health officer's side will be presented by Dr. A Wadsworth, State Department of Health, New York.

A separate committee will draft recommendations for each of the four sections for presentation to the assembly. The respective chairmen of these four section meetings will be Drs. Walter M. Simpson, Dayton, Ohio; Arthur H. Sanford, Rochester, Minn.; F. E. Senear, Chicago, Ill.; and H. H. Hazen, Washington, D. C. General discussion will follow the presentation of each set of recommendations.

An additional feature of the meeting will be an actual demonstration of the performance of the Eagle, Hinton, Kahn, Kline, and Kolmer tests by the originators of these procedures.

It is to be hoped that the attendance at this assembly will be large. Out of the meeting should come a crystallization of opinion with regard to the im-

portant problems which will be considered. Those interested in obtaining further information should write to the Surgeon General, U. S. Public Health Service, Washington, D. C.

The program follows:

I. Aims and purposes of the assembly. DOCTOR THOMAS PARRAN.

II. Need for adherence to the conventional technic in the routine performance of reliable serodiagnostic tests for syphilis.

Recent significant changes in the technic of the Eagle complement fixation and flocculation tests. DR. HARRY EAGLE, Johns Hopkins Hospital, Baltimore, Maryland.

Recent significant changes in the technic of the Hinton flocculation test. DR. W. A. HINTON, State Department of Health, Boston, Mass.

Recent significant changes in the technic of the Kahn presumptive and standard diagnostic flocculation tests. DR. REUBEN L. KAHN, University of Michigan Hospital, Ann Arbor, Michigan.

Recent developments relating to the slide tests for syphilis. DR. BENJAMIN S, KLINE, Mount Sinai Hospital, Cleveland, Ohio.

Recent significant changes in the technic of the Kolmer complement fixation test. DR. JOHN A. KOLMER, Temple University, Philadelphia, Pennsylvania.

Frequent errors noted in the performance of serologic tests. SENIOR SURGEON J. F. MAHONEY, Venereal Disease Research Laboratory, Staten Island, New York.

Recommendations of Committee on need for adherence to conventional technic. Chairman: DR. WALTER M. SIMPSON, Maimi Valley Hospital, Dayton, Ohio.

General Discussion (discussants limited to five minutes).

III. Training of Laboratory Personnel.

Minimum qualifications for laboratory directors engaged in the performance of serodiagnostic tests for syphilis. DR. ARTHUR H. SANFORD, Mayo Clinic, Rochester, Minnesota.

Need for the training of technicians in the performance of serologic tests for syphilis. DR. A. S. GIORDANO, South Bend, Indiana.

Recommendations of Committee on Training of Laboratory Personnel. Chairman: DR. ARTHUR H. SANFORD.

General Discussion (discussants limited to five minutes).

IV. Demonstration of the Eagle, Hinton, Kahn, and Kolmer methods of serodiagnostic tests for syphilis by the originators. Senior Surgeon J. F. MAHONEY in charge of demonstrations.

V. The prosecution of studies of the efficiency of the performance of serologic tests for syphilis within the States.

Satisfactory specificity and sensitivity ratings. DR. H. H. HAZEN,

Professor of Dermatology and Syphilology, Howard University, Washington, D. C.

The relative value of doubtful reports in conducting evaluation studies of serodiagnostic tests for syphilis. DR. F. E. SENEAR, University of Illinois, Chicago, Ill.

The advantages of reporting serodiagnostic tests as positive, doubtful and negative. DR. JOSEPH EARLE MOORE, Johns Hopkins Hospital, Baltimore, Maryland.

Evaluation of serologic tests in Maryland. DR. C. A. PERRY, State Department of Health, Baltimore, Maryland.

Evaluation of serologic tests in Cleveland, Ohio, DR. ROBERT N. HOYT, Cleveland Health Council, Cleveland, Ohio.

Dried serum used in the evaluation of serodiagnostic tests by the New Jersey State Department of Health. DR. A. J. CASSELMAN, State Department of Health, Trenton, N. J.

Recommendations of Committee on Methods for Improvement of Studies to Determine the Efficiency of Serologic Test Performance. Chairman: DR. F. E. SENEAR.

General Discussion (discussants limited to five minutes).

VI. The desirability of State departments of health approving or licensing laboratories for the performance of serodiagnostic tests for syphilis.

From the standpoint of the State health officer, DR. A. WADSWORTH, State Department of Health, Albany, New York.

From the standpoint of the private laboratory director, DR. FREDERICK H. LAMB, Davenport, Iowa.

Recommendations of Committee on licensing or approval. Chairman: DR. H. H. HAZEN.

General Discussion (discussants limited to five minutes).

BOOK REVIEWS

Introduction and Guide To The Study of Histology. By AVERY E. LAMBERT, PH.D., Professor of Histology In The School of Medicine, State University of Iowa. Cloth, 542 pp., 185 figures. P. Blakiston's Son & Co., Philadelphia, Penna.

This volume is intended to be essentially a student's working text to furnish the material and knowledge of a working knowledge of histology. As such, it may confidently be expected to be successful and fill a need, for it is both well organized and clearly written. Generously illustrated with line drawings and microphotographs, the physician as well as the student may use it with profit.

Without doubt this will be a popular book.

The Big House of Mystery. By PATRICK H. WEEKS, M.D., Physician and Psychiatrist, Indiana State Prison. Cloth, 259 pp., 5 illustrations, $2.00. Dorrance & Co., Philadelphia.

This is not only a very interesting but a very instructive book. The author, from an extensive experience has gained a clear insight of crime and the criminal and has written from his experience a careful, thoughtful and constructive book.

It may be read as a story or as a scientific contribution to the study of the criminal. In either event it will be read with interest and profit.

A Textbook of Pathology. Edited by E. T. BELL, M.D., Professor of Pathology, University of Minnesota. Ed. 3, Cloth, 894 pp., 412 illustrations, 2 colored plates. $9.50. Lea and Febiger, Phila.

This third edition of Bell's well known text has been extensively revised and expanded with the addition of 67 new illustrations.

This is a book not only for the student but useful also to the physician because of its eminently practical approach, and the correlation of pathological phenomena with clinical medicine. As before, this edition may be recommended as an excellent text for the physician, the student and, indeed, for all who are concerned with the phenomena and manifestations of disease. The pathologist will find it a useful addition to his reference library.

Leukemia And Allied Disorders. By CLAUDE E. FORKNER, A.M., M.D., Assistant Professor of Clinical Medicine, Cornell University Medical School. Cloth, 333 pp., 73 figures, 6 colored plates. The MacMillan Co., New York.

Although in the nearly one hundred years which have passed since the first description of leukemia the literature on the subject has increased tremendously, the disease remains a puzzle and a problem.

In this book—one of the MacMillan Medical Monographs—Dr. Forkner presents a comprehensive and systematic survey of all that is known of leukemia. Not only the clinical and hematologic aspects of the disease are discussed in the light of a correlated and coördinated survey of the literature of leukemia, but also there is a scholarly and critical discussion of the fundamental aspects of the problem and particularly of those phases concerned with the pathological physiology of the hematopoietic system.

A comprehensive bibliography and an extensive author index are included.

Neither the physiologist, pathologist, hematologist, nor physician can afford to miss this book.

It contains perhaps the most comprehensive and authoritative discussion of leukemia yet printed and without doubt will be a classic in its field.

Diabetes Insipidus and The Neuro-Hormonal Control of Water Balance: A Contribution To The Structure And Function Of The Hypothalamico-Hypophyseal System. By CHARLES FISHER, PH.D., W. R. INGRAM, PH.D., and S. W. RONSON, M.D., Institute of Neurology, Northwestern University Medical School. Cloth, 212 pp. Edwards Bros., Inc., Ann Arbor, Michigan.

This book presents the results of an intensive investigation into the nature and structure of the mammalian hypothalamus as a result of which the authors have formulated a concept of the various factors concerned with the regulation of water balance and the cause of its disturbance in diabetes insipidus.

Their work leads them to support the view that diabetes insipidus is essentially a hormonal disturbance and that the polyuria in this disease represents the results of diuretic processes unchecked by the antidiuretic mechanism.

This is perhaps one of the most comprehensive discussions of the subject yet available and should prove of great interest to the physiologist, physician and all who are interested in this disease.

Syphilis, Gonnorrhea, And The Public Health. By NELS A. NELSON, B.S., M.D., F.A.P.H.A. Director, Division of Genito-Infectious Diseases, Massachusetts Department of Public Health, and GLADYS L. CRAIN, R.N., Epidemiologist, Division of Genitoinfectious Diseases, Massachusetts Department of Public Health. Cloth, 359 pp., 8 illustrations. $3.00. The MacMillan Co., New York.

It is to be expected that the present interest in syphilis will result in numerous books intended for the education of the public. It is to be regretted that not all of them will be as sane and as truly suited for popular consumption as this book.

The authors are thoroughly familiar with their subject, have quite obviously had ample experience, and present their story in logical sequence, clearly and with a welcome absence of bombast and rhodomontade. The laity who read this book will gain a clear understanding of the problem as well as of its inherent

difficulties. The health officer, the physician and all who may be called upon to discuss it before lay groups may read this book with profit. It can be recommended as a useful contribution.

A Symposium On Cancer. Cloth, 202 pp. $3.00. The University of Wisconsin Press, Madison, Wisconsin.

In this book are presented seventeen addresses given before an Institute On Cancer conducted by the Medical School of The University of Wisconsin.

That the discussions are authoritative is obvious from the list of contributors: Leiv Kreyberg, Clarence C. Little, Madge T. Macklin, Edgar Allen, Howard B. Andervont, James Ewing, Gioacchino Failla, Henri Coutard, Warren H. Lewis, Stanley P. Reimann, James B. Murphy, and Emil Novak.

These names are familiar to all who have followed the literature concerned with cancer from both the experimental and clinical angles. For this very reason none who are interested in cancer in all its varied aspects can fail to be interested in this book which presents in interesting fashion the present status of scientific knowledge about cancer. The biologist, biochemist, physiologist, pathologist, and practitioner alike may well add this book to their reference library to be read with interest and profit.

Crime, Crooks And Cops. By AUGUST VOLLMER, Professor of Police Administration, University of California, and ALFRED E. PARKER. Cloth, 260 pp. $2.00. Funk and Wagnalls Co., New York.

August Vollmer has long been known as one of the foremost exponents of scientific methods in the study and detection of crime. In this book he presents in non-technical and popular fashion the various laboratory and other methods now used in detection, illustrating their use by the recital of various cases. The book is not only informative but makes interesting reading and discusses crime and police problems with understanding and candor.

A Manual of The Common Contagious Diseases. By PHILIP MOEN STIMSON, M.D., Associate Professor of Clinical Pediatrics, Cornell University Medical College, Ed. 2, Cloth, 437 pp., 53 illustrations, 3 plates, $4.00. Lea and Febiger, Philadelphia.

That this compact, concise yet comprehensive volume has reached a second edition will not be surprising to those familiar with it nor is there much doubt that this book will continue to remain a favored text in its field.

Whether consulted by the student, the practitioner desiring a rapid review of a specific subject, the health officer, interne, school or industrial physician, this volume will be found adequate. The subject is well and clearly presented and in an eminently satisfactory manner.

Dr. Stimson's book can be highly recommended.

Textbook of Experimental Surgery. By J. MARKOWITZ, Research Associate, Department of Physiology, University of Toronto, Formerly Professor of Physiology, Georgetown University School of Medicine; Formerly Assistant in Division of Experimental Surgery and Pathology, Mayo Foundation, Rochester, Minn. Cloth. 527 pp.; 330 figures. $7.00. WILLIAM WOOD & CO., Baltimore.

As Dr. Balfour says in the foreword to this book, not only have the surgical advances of the last half century been largely the aftermath of laboratory experimentation, but experimental surgery has now become essential to an adequate postgraduate training.

"This volume has for its purpose the presentation of some of the major accomplishments of experimental surgery and the demonstration in some detail of the technical procedures by which this knowledge was acquired."

Space does not permit the tabulation of the various procedures described in detail; but it can be said that there is no type of surgical approach, nor any area of the body which is not represented.

The style is easy and clear; the illustrations numerous and truly illustrative. By the embryo surgeon, the postgraduate student and to the investigator this book will be heartily welcomed as a contribution of distinct value and utility.

Medicolegal Aspects of The Ruxton Case. By JOHN GLAISTER, M.D., D.SC., Barrister-at-Law, Regius Professor of Forensic Medicine, University of Glasgow, and JAMES COUPER BRASH, M.D., M.A., F.R.C.S., Ed., Professor of Anatomy, University of Edinburgh. Cloth, 284 pp., 172 illustrations. $6.00. WILLIAM WOOD & CO., Baltimore.

Professor Glaister has achieved an international reputation as an expert in medicolegal investigations and his textbook on Legal Medicine can be found in the libraries of the world at large. Varied as has been his experience, it is doubtful if it has heretofore encountered as bizarre and complex a problem as that presented by the Ruxton case, the medicolegal aspects of which are here related in detail.

This is, indeed, an intriguing book concerning a case in itself unique.

On the afternoon of September 29, 1935 the police of a small town near Dumfriesshire, Scotland, were notified of the presence of human remains in a ravine below a bridge on the Moffat-Edinburgh road. Four bundles containing parts of two human bodies were found that day and others on later and more extended search, the last piece being discovered on November 4 following a heavy storm which had blown away the dead bracken under which it lay.

In all some seventy-seven pieces were found representing two human bodies which had not only been dismembered but had undergone extensive dissection and mutilation with the obvious purpose of preventing identification.

Further investigation developed that they were two women, the wife and servant of Dr. Buck Ruxton, and he was charged with their murder.

The problem thus presented to the medicolegal experts for the Crown was thus one of extraordinary and even unprecedented difficulty. This book relates in detail the methods used and the manner in which they were applied to the reconstruction and identification of the bodies, as well as the other medicolegal investigations utilized in the case.

As commented upon in the preface, this case "is notable primarily because the extent and character of the mutilation of the two victims provided a problem of reconstruction which demanded for its solution anatomical work in detail not hitherto required in such cases, and because the purposive removal of identifying features suggested a novel comparison of skulls and portraits which, with other circumstantial evidence, helped to place identification beyond doubt."

All the varied phases of forensic medicine and other specialized fields that were required are described in full.

The illustrations are extraordinary and superbly reproduced.

This is a book of great interest and value to the pathologist who includes medicolegal investigations among his activities; to the lawyer; to police authorities and, indeed, even to the general medical reader as an absorbing illustration of the varied and ingenious methods applicable to medicolegal investigations.

Pneumonia And Serum Therapy. By FREDERICK T. LORD, Clinical Professor of Medicine, Emeritus, Harvard University and RODERICH HEFFRON, Field Director, Pneumonia Study and Service, Massachusetts Department of Public Health. Revised Edition of Lobar Pneumonia and Serum Therapy. Cloth, 148 pp. 10 figures, $1.00. The Commonwealth Fund, New York.

Even in the short time elapsing since the appearance of the first edition (1936) progress in the field of serum therapy in pneumonia has necessitated extensive revision of this book.

The change in title reflects the newer knowledge that serum therapy is applicable to bronchopneumonia and atypical forms as well as to lobar pneumonia.

Among the more important additions and changes are further data on the incidence of pneumococcus types in pneumonias, case fatality rates; data on the result of serum treatment in a larger series of Type I and II pneumonia and in cases due to certain of the higher types; and an outline of basic plans for a pneumonia control program.

Changes in dosage (Type I, at least 60,000 units; Type II, at least 100,000 units, in divided doses; an initial dose of at least 60,000–100,000 units in Types VII, VIII and XIV) are recommended and the use of rabbit serum is discussed.

As before this book is almost indispensable to the physician's library.

THE DESIRABILITY OF STATE LICENSURE (OR NATIONAL APPROVAL) FOR LABORATORIES PERFORMING VENEREAL DISEASE TESTS*

(Incorporating More Than 300 Replies to a Questionnaire on This Subject)

FREDERICK H. LAMB

Davenport, Iowa

The complex question of licensing laboratories or laboratory personnel to perform serological and bacteriological tests as related to the control of venereal disease presents a host of individual problems. Some of these pertain to public welfare, some are of a scientific, others are of administrative and legal nature, while still others are economic problems. It seemed to me that discussion of various phases of this subject by a large number of those who are vitally interested might contribute something to a better understanding of these problems. With this end in view, I shall present an analysis of more than 300 replies to a questionnaire.

One or two of the questions should have been more explicit in nature, and replies to several additional questions would have been enlightening; nevertheless the responses as a whole were so complete and comment so abundant that it was not difficult to gauge the writer's reactions.

It should be understood that it is the purpose of the questionnaire and of this paper to deal with the practical aspects of this problem in a practical manner. One is not concerned here with merely abstract or philosophical ideals, nor is this the occasion to discuss any such technical matters as native reagins in blood

* Presented at the annual meeting of the American Society of Clinical Pathologists, San Francisco, Cal., June 10–11, 1938. Received for publication, August 21, 1938.

sera as related to the specificity of serologic tests for syphilis. This is an attempt to analyze and correlate the reactions of men and women of this country who have been making laboratory examinations for venereal disease as a daily routine for from 5 to 25 or 30 years. These are the people who would be more affected by a system of licensure such as that under discussion, if it came to pass, than any other professional group. These are the people whose cooperation or lack of it would accelerate or retard the effectiveness of any such system.

Beginning with the three questions on which there was the least difference of opinion, I shall repeat the question and summarize the discussions in the succeeding paragraphs.

1. Question 2 of the questionnaire was: *"Should a laboratory be licensed as a business concern, or should the individual personnel be licensed?*

Replies to the question were overwhelmingly in favor of individual licensure, if any, and were tabulated as follows:

Individual	261
Business concern	12
Neither	27
No opinion	6

There was a general agreement that reliability and integrity of laboratory examinations depended on personnel rather than equipment, business standing, or even traditions of the laboratory. In most of the comments favoring licensure it was emphasized that disreputable laboratories and incompetent personnel could in this manner be eliminated. Comments from those who doubted the wisdom of any form of licensure, or who were positively opposed to it, centered about two points: first, that licensure might permit an increase of irresponsible lay-supervised laboratories; and second, that no additional form of license was necessary. Not a few opponents to licensing contended that ample approval of laboratory directors was implied by their membership in the American Association of Pathologists and Bacteriologists, the American Society of Clinical Pathologists, or by certification of the American Board of Pathology. The same form of approval, it was held, applied to medical technologists through their registration.

2. Question 3 of the questionnaire was: *"Should the operation of laboratories be considered as a branch of the practice of medicine?"* Replies to this question were all but unanimously in the affirmative as shown below:

Affirmative	297
Negative	5

Some declared that a positive answer to this question was too obvious to merit any discussion, although the great majority noted the important relationship between this and the other questions. It is quite as fundamental as the question of licensure itself.

3. Question 6 of the questionnaire was: *"Do you believe there there is an advantage in having a number of qualified or licensed laboratories throughout a state, rather than one or more central laboratories?"* Here again there was a general agreement in that the majority of replies favored the decentralization of laboratory facilities as indicated in the following tabulation:

For multiple laboratories	286
For central laboratories	15

It was brought out in the commentary discussion that delays in transportation and innumerable reports of damage to specimens, together with the very questionable practice of the sending of work from home that could just as well be done at or near home, far outweighed the advantages of centralization. It was pointed out that local laboratories, with all the advantages of prompt reports, dark fields, prompt spinal fluid examinations and multitudinous other services, would increase in number if a reasonably fair revenue were permitted to accrue to the support of the local laboratory. In the few sparsely populated states, a central state board of health laboratory is probably the only solution of the problem, while in densely populated centers there is probably no need for more laboratories. But in the great majority of states expansion of laboratory facilities is urgently needed. There is no doubt that the policy of state and municipal laboratories in performing not only serologic tests, but all sorts of laboratory examinations whether related to public health or not, without the slightest regard for the patients' economic

status leads to abuses which need to be controlled. For one thing, *the patient in numerous instances pays for a test, but the fee does not reach the laboratory.* If there is to be any system of licensing, this sort of thing must be rooted out.

4. Question 9 of the questionnaire was: *"Should the work of clinical laboratories be checked from time to time by unknowns or inspection?"* There was somewhat less agreement among the replies on this question as shown by the following tabulation:

For checking	203
Against checking	90

The principal objections to inspection came from those who objected to any form of license, registration, or approval, although not a few who objected to licensure favored checking by unknowns. The majority favored one system or both for checking results of tests, some advocating it strongly as a means of reducing unreliable or careless work.

A suggestion which appealed to the writer as being effective and uncomplicated was noted in several responses, namely, that if lay-operated laboratories were eliminated, and laboratories under medical supervision were checked from time to time by unknowns or by inspection or both, most of the problems of control and licensure would be solved as simply as that.

5. Question 10 of the questionnaire was: *"Should antigens be furnished by a central or state laboratory?"* The majority favored the trial of this innovation chiefly as a step leading toward a greater uniformity of results.

In favor	188
Against	103
Noncommittal	7

Again the principal objections came from those who looked with disfavor on any additional form of license or control. On the other hand, some who objected to licensure, advocated the use, principally for control purposes, of a uniform antigen. Assuming that antigens to be supplied in this way would be checked most carefully before distribution and that they would be avail-

able free or at a nominal cost, the advantages seemed to far outweigh any of the specific objections mentioned.

Question 7 of the questionnaire was: *"If you were to serve on a state licensing board, what important qualifications for licensure would you advocate?"* and question 8 was: *"From the stand point of a director of a private or hospital laboratory, what safeguards would you advocate against abuses or excesses of power or jurisdiction on the part of the licensing board?"* Both of these questions were thoroughly discussed, and since they are in a sense complementary, comment on them may be combined. Two points were especially clear. First, since the diagnosis of syphilis, as that of other diseases, constitutes the practice of medicine, a laboratory should be under the active supervision of a legally responsible physician trained and qualified by experience in laboratory work; and second, political influences must be eliminated. It was repeatedly emphasized that the activities of a licensing board should be limited strictly to venereal disease work. Those opposed to any form of licensure of course saw no reason whatever for licensing boards and many vigorous warnings against political abuses were noted among the replies. Many comments on these two questions applied more directly to the question of licensure as a general proposition and will be dealt with later under that heading.

Question 5 of the questionnaire was: *"As a partial recompense for the voluntary qualifying of a laboratory for license should subventionary financial assistance be rendered to it?"* This question should have been stated more explicitly, but the replies, as the question stands, were as follows:

For subventionary financial assistance	170
Against subventionary financial assistance	93
Replies with qualifications and reservations	40
Noncommittal	3

There was a substantial difference of opinion on this question. Its true intent was twofold: first, should a private or hospital laboratory, if it qualifies for license, be paid from public funds for the performance of venereal disease tests on indigent pa-

tients? Second, should a qualified or approved laboratory be paid for the performance of all venereal disease tests from public funds, since from a standpoint of venereal disease control, it renders a public service just as certainly as does the state or municipal laboratory.

In many replies a distinction was made between work done for private patients and for indigent cases and wards of the state. Most replies advocated the payment from public funds covering indigent cases. A small minority, with which this writer agrees, suggested that since the venereal disease control campaign was a public service, all related laboratory tests should be paid for on that basis and from public funds. On the other hand, many strongly worded objections to subsidies were based on the assumption that this was simply an entering wedge to secure control of all laboratory work as another step in the advancement of state medicine. Not a few vehement denunciations of this trend were noted.

Question 1 of the questionnaire was: *"As a part of the National and State Antivenereal Disease Campaign, do you believe any form of licensure desirable (for the performance of serological and bacteriological tests)?"*

Question 4 of the questionnaire was: *"Should licensure become compulsory or be voluntary?"* Since the latter question is to some extent a corollary of the former, the discussion has been combined.

An analysis of the responses to these questions was tabulated as follows:

In favor of licensure	166
Against any form of licensure	107
In favor of approval (or registration)	31
Noncommittal	13
In favor of compulsory licensure	150
In favor of voluntary licensure	74
In favor of voluntary approval	64

Comments on and reasons given for responses to these questions were far more instructive as to the correspondents attitude than the "yes" or "no" answer. For example, it was

apparent that many who advocated licensure did so with reservations. Some who voted for licensure really favored approval or registration, but preferred license to no control at all. The great majority who favored the licensing of laboratories for the performance of venereal disease tests took the stand that this is the most practical and possibly the only practical means of eliminating the incompetent and unreliable laboratory. Not a few expressed the idea that the character of all laboratory work would be improved through a system of licensing. Several replied that any medical procedure that is so widely recommended to the public should have the state's sanction and assurance of reliability.

A group of replies comprising about 10 per cent of the whole favored licensing, or more strictly speaking, approval by the American Medical Association as at present or by one of the National Medical Societies such as the American Society of Clinical Pathologists. Without exception these correspondents, and many others, objected to licensing by any political body.

The most vehement objections came from fully one-third of the correspondents who were strongly opposed to any new or additional form of licensure, approval or registration. Why single out the clinical pathologist or the laboratory for special licensure? Does not the license to practice medicine cover the operation of the medical laboratory? Why not eliminate and proscribe the operation of laboratories not under the supervision of licensed physicians? Laboratory licensure would thus become automatic, just as any other branch of medical practice is automatically under license. These and other pointed questions were frequently encountered. And so far as my information goes, satisfactory forthright answers are not readily forthcoming from any source.

SUMMARY AND COMMENT

It is quite impossible to summarize all of the divergent views which have been expressed. There is a substantial agreement on certain phases of the problem. For example, it is the consensus of the great majority that if any system of licensure, regulation,

approval or registration is to be promulgated, it should among others embrace the following objectives: First, it should apply to the individual and not to a commercial or business concern. Second, it should proscribe the non-medical lay operated laboratory as having no place in the practice of medicine. Third, it should extend recognition and sanction to all qualified laboratories throughout a state in order to render laboratory service more readily available, and to offset the disadvantage of centralization. Fourth, it should eliminate inequality and downright petty graft.

As to the heart of the question itself, that is, the desirability of licensing or approving laboratories for the performance of tests related to the control of venereal disease, replies to the questionnaire indicate profound differences of opinion. It is only fair to state that in reading through the responses one after another, a plea for improvement in existing conditions was frequently encountered. As an indication of a tolerant and comprehensive understanding, many to whom the principle of license was frankly distasteful, expressed a willingness to comply with regulations if that proved to be the only consistent manner of elevating standards and eliminating the unfit. Thus, while there was no attempt to evade or play up the issue of public welfare, it can not be said that this phase of the question was wholly ignored. Undoubtedly, the question of public welfare will be hailed as the paramount issue by the proponents of licensure both within and outside the medical profession. It is bound to be the avowed motive for any statutory program which may be launched.

In the writer's opinion it is not unlikely that some system of regulation will be evolved, ostensibly in the public interest. Evaluation studies of serodiagnostic tests for syphilis have demonstrated a surprising lack of specificity and sensitivity not only of certain serologic methods, but a lack of uniform results with the same method. Moreover these investigations have not thus far been extended to laboratories where the greatest discrepancies might be expected to exist. It is probably safe to say that an allowable error of 1 or 2 per cent in specificity and

10 to 20 per cent in sensitivity as compared with a control laboratory is being exceeded far too frequently. It can not be denied therefore that there is a strong case for some form of systematic improvement and regulation.

Such regulation may be projected along either of two lines, first a system of state licensure; second a system of national approval on a voluntary basis. There is the third possibility that a state might exercise its licensing power as an adjunct to national approval. New York State, Michigan, and California have already enacted statutory regulations within the framework of the medical practice acts of their state legislatures. Indeed, all existing state examining and licensing boards operate under this authority by virtue of what is known as administrative law rather than by individual statute. It seems to be generally understood that while a legislature cannot delegate its legislative powers to a subordinate body, nevertheless, the courts have recognized that a legislature can delegate to an official body the duty of putting in details under the law that are necessary to the accomplishment of some general purpose set out in a statute. Thus, the most steadfast opponent to licensure must recognize that so far as state laws are concerned, it is only a short step to mandatory regulation. The individual protest is after all only an individual protest.

Without attempting any farther excursion into the administrative or legal aspects of this problem, would it not be the part of wisdom for members of this society and others vitally interested to recognize that there is, in this era of social unrest, a trend toward statutory regulation. As this trend prevails and materializes, if no better solution of the problem can be found, then we must be prepared to guide and mold it to the end that all inequalities, weaknesses, and failures existing under present conditions be recognized and remedied so far as that is possible. In countless instances the most glaring faults are currently quite beyond the power of the individual clinical pathologist to remedy.

On the other hand, while a state may have the legal machinery to license, there is evidence that it may not have what it takes

to evaluate the work of the medical laboratories within its borders. Moreover, one must be naive, indeed, to believe that such delicate qualities as care, accuracy and reliability can be legislated into any scientific procedure. These are not commodities, they are the attributes of scientific attainment and must be accepted as such. Mandatory regulation, therefore, through a system of state licensure leaves much to be desired from a scientific standpoint.

The other alternative, namely, a system of national approval on a voluntary basis has possibilities which are worth exploring. First, the problem in hand is national in scope and should be attacked from a national vantage point. Second, the reward of national recognition for meritorious service should provide an incentive for a high standard of work. Third, precedent exists in the form of successful standardization of medical schools and hospitals through the exercise of moral suasion on a national scale.

The writer does not pretend to have all the answers for such a plan. Its inauguration will require, time, study, education, cooperation and leadership. Preliminary investigations might be undertaken by a committee of the U. S. Public Health Service functioning in the same manner as the Committee on Serologic Evaluation Studies. Eventually a National Board of Registration and Approval, free from political influence and of unquestioned professional and scientific standing, might be empowered to administer the plan. If tactfully and successfully executed, such a plan as this should in time establish laboratory diagnosis, in the control of venereal disease, on a high standard of efficiency.

As to a decision between voluntary approval and compulsory approval or licensure, the writer is open to conviction so far as state and municipal tax supported laboratories are concerned, but in the case of hospital and private laboratories the application for approval or licensure on the part of the laboratory should be voluntary. Unless the government is prepared to pay hospital and private laboratories an adequate recompense for quali-

fying and performing tests there should be no question of compulsion, other than the license to practice medicine.

CONCLUSION

Since the inauguration of a nation-wide laboratory service free to physicians (but not always so to patients) through the greatly augmented activities of tax supported state and municipal laboratories, there has been a marked reduction in the volume of serologic work in private and hospital laboratories. The inevitable result has been to reduce expense and curtail or eliminate the local service altogether. Frankly, this trend is not likely to recede for it is impractical to maintain a Wassermann system, and it is unsatisfactory to run a flocculation system merely for the emergencies that arise. Certainly, such a trend is prejudicial to an efficient service due to delays in the transportation of specimens and reports, and unsatisfactory spinal fluid and dark field examinations, to say nothing of pre-transfusion immunological tests and other emergencies where laboratory evidence for or against syphilis should be quickly available. Thus, the centralization of laboratory facilities may have its advantages in large population centers, but the disadvantage and penalties of eliminating local service are becoming more and more apparent elsewhere.

Finally, and with no little reluctance, it must be admitted by those in close touch with private and semi-private laboratory services, these trends have already cast their shadows. Enthusiasm gives way in time to indifference, when the end no longer justifies the means. Further developments along these lines, therefore, may preclude the necessity for state licensure or national approval in a number of states and in many localities, except as one plan or the other is desirable for state and municipal laboratories.

CORRELATION STUDIES OF BASOPHILIC AGGREGATION AND RETICULOCYTES IN VARIOUS CLINICAL CONDITIONS*

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Since Askanazy's[1, 2] initial studies on reticulated red cells, others (Keyes,[3, 4] Cunningham,[5] Seyfarth,[6] Pappenheim, Isaacs,[7] and Ferrata[8]) have contributed findings which have somewhat clarified the significance, properties[9] and characteristics of reticulocytes in the peripheral blood stream. The result is today's widely accepted concept that young erythrocytes manifest themselves on the stained slide as polychromatophilic, stippled, or reticulated forms depending on the amount, distribution, and state of the basophilic reticulum within the cell. The duration of contact between the stain and the basophilic material is important for the demonstration of these immature cells, as is seen by the invariably higher reticulocyte counts which are secured when a "wet" method of staining them is used (the blood being held in longer contact with the stain as a solution), as compared with "dry" methods.[10] This doubtless accounts for the varying normal values of reticulocytes (0 to 4.0 per cent) in the literature.[10]

The nature of the basophilic reticulum in the red cell which may assume a polychromatophilic, stippled, or reticulated appearance is not proven. It occurs in the normal process of maturation of the erythrocyte. Three origins have been suggested. One proposed by Naegeli and Seyfarth[6] considers the material a product of nuclear decomposition. Keys and Dawson recently considered a genetic relation of the reticulum network to mitochrondia. The most widely accepted concept today considers the basophilic reticulum as "retained undifferentiated cytoplasmic

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basophilic material of immature ontogenetic forestages of the red cell series (erythroblasts)."[31]

Accompanying the advancements made, many workers have found themselves seeking these young cells as diagnostic and prognostic indicators in various conditions, as pneumonia, tuberculosis, plumbism, and others.[11, 12, 13, 14, 15, 16, 17, 18] Knowingly or otherwise, some have ignored the fundamental fact that the number of reticulated cells circulating in the blood is merely an index of bone marrow regeneration or erythropoetic stimulation. Any toxin which inhibits this cellular regeneration will depress the reticulocyte level and, conversely, any erythrogenetic substance (liver extract) or situation, (secondary anemia, anoxemia) will elevate it. Certain weather factors and vitamin C[19] also seem to exert this influence.[20] It is this stimulating or inhibiting factor alone, and not disease entities, per se, which must be considered.

McCord,[21, 22, 23] in 1924, introduced a new method for staining blood smears which he called the "Basophilic Aggregation Test" which utilizes an alkaline stain (Methylene blue) in a hypotonic solution. This method was, and still is, proposed primarily as an industrial measure to detect early cases of lead absorption and lead poisoning. The stress put upon this purpose has associated this procedure of staining blood smears with the lead hazards. Jones[24] has shown that the "Basophilic Aggregation Test" gives results that correspond and correlate with the usual reticulocyte "vital" staining methods. The presumption is therefore great that the "Basophilic Aggregation test" is simply another reticulocyte staining procedure.

On many occasions, we have been called upon to determine the presence or absence of lead intoxication in patients hospitalized with a history of exposure to lead. The validity and significance of the basophilic aggregation counts of necessity had to be evaluated along with other clinical and laboratory findings in reaching a conclusion. Since this problem is of such scientific and medicolegal importance it was considered desirable to reinvestigate this field and to study the relationship of basophilic aggregations to reticulocytes.

Plan of investigation

In this investigation we have studied (a) the reliability and limitations of the "Basophilic Aggregation Test," and (b) the correlation between basophilic aggregations and reticulocyte counts. The subjects used consisted of five normal individuals, forty-six patients presenting a wide variation in bone-marrow activity and two rabbits intoxicated with lead.

Methods

General hematologic studies were made on all patients as required for purposes of diagnosis. These studies included: Wintrobe indices,[25] red and white cell counts, differentials, and sternal marrow aspirations.[26]

To stain the reticulocytes, we utilized brilliant cresyl blue and followed a staining procedure which is a modification of Cunningham's "vital" staining method.[5] A clean cover-slip is covered with a thin film of 0.5 per cent alcoholic solution of brilliant cresyl blue which is allowed to dry. This dried surface is then gently rubbed over a smoothly-grossed paper surface to rub off excess stain until a violet cast is evident. Many such preparations may be so made and kept indefinitely. A small drop of the patient's blood is collected on the edge of a clean, unstained coverslip and carried to the previously stained coverslip where it is mixed with the stain present until it turns a dirty greenish blue color, after which both slips are opposed and the blood "sandwiched" and spread between them. The slips are then immediately and quickly drawn apart. Both coverslips are finally dried by air in motion, and stained with Wright's stain as usual. It is possible, however, to put these smeared coverslips aside and stain with Wright's stain as late as a week afterwards. The stained and dried slips may be then mounted on slides and counted.

The "Basophilic Aggregation Test" was performed according to McCord's method,[21, 22, 23] which utilizes Manson's borax-methylene blue stain. Jones'[24] modification which fixes a longitudinal strip of the blood smear was also adopted.

All counts were made by using a Whipple grid in the microscope ocular piece and counting not less than 500 cells from scattered fields. (A palm-sized tabular helps greatly during the counting procedure.)

RESULTS

Reliability and limitations of tests

After two years of experience with the basophilic aggregation test, we can not substantiate the claim of simplicity which has been made for it. We have found that such factors as: (1) time limits, (2) stains, and (3) microscopic identification of basophilic aggregation cells all lead to variable results.

(1) McCord[21, 22, 23] and Jones[24] have pointed out the time

limit of several hours which should not be exceeded before the slide is stained. Our experience bears this out for we have found that only one day's passage reduced the counts of slides from 12.1 per cent to 9.0 per cent; 24.6 per cent to 19.2 per cent—this last count falling to 1.6 per cent in five days. This can be attributed to drying effects and reticulum degeneration.[6]

TABLE 1

Basophilic aggregation counts made by different observers

Basophilic aggregation counts

SLIDE	OBSERVERS			AVERAGE	PER CENT VARIATION
	A	B	C		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	10.0	8.2	4.1	7.4	44
2	11.4	7.3	8.1	8.9	28
3	4.2	0.8	1.5	2.2	91
4	8.4	4.1	6.4	6.3	35
Average variation					49.5

Reticulocyte counts

SLIDE	OBSERVERS			AVERAGE	PER CENT VARIATION
	A	D	E		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
5	7.0	8.2	8.5	7.9	11.4
6	8.5	9.0	8.3	8.6	4.6
7	26.7	27.8	33.0	29.2	13.0
8	37.3	38.6	30.0	35.3	15.0
Average variation					11.0

Note: Observers B, C, D, and E were selected because of their experience and proficiency in performing the respective counts.

(2) McCord[21, 22, 23] has already pointed out the non-uniformity of the Sussman-Weidel stain (which Jones recommends) and Jones,[24] has mentioned the non-uniformity in staining power of Manson's methylene blue stain (which McCord recommends). We are in accord with both criticisms. Precipitation of the Manson stain proved to be a serious handicap.

(3) Only after prolonged experience studying cells stained

according to the basophilic aggregation method can one arrive at consistent results. Even then, we noted that a large personal difference existed between counts made by different individuals with the same amount of experience. In fact, the same individual finds it difficult to obtain acceptable duplicate counts, when the same slide is recounted. For this reason, it was deemed better to allow only one worker to perform all basophilic aggregation counts which are presented in this report. The reason for the discrepancy in counting results between individuals is the fact

TABLE 2

Basophilic aggregation counts made by same observer

SLIDE	OBSERVATIONS		AVERAGE	PER CENT VARIATION
	First	Second		
Basophilic aggregation counts				
	per cent	*per cent*	*per cent*	*per cent*
9	0.2	0.6	0.4	50.0
10	7.1	9.6	8.3	15.6
11	2.3	3.3	2.8	18.0
Average variation				27.8
Reticulocyte counts				
12	0.5	0.6	0.5	9.0
13	8.0	8.3	8.1	1.8
14	11.2	11.9	11.5	3.0
Average variation				4.6

that absolute identification of the basophilic aggregation cells is not readily accomplished. The cells have been morphologically altered to "cell shadows" which may be and are simulated by particulate artifacts which are always present (precipitated stain, organic matter). The presence of many lymphocytes in the blood smear has also led to erroneous counts by an experienced worker using this stain.

Since Jones finds the basophilic aggregation method more accurate, it was deemed necessary to reinvestigate the reliability

of this test as compared with our modified reticulocyte method. Jones,[24] secures a coefficient of variability by "taking the deviations of individual counts from the mean for all readings made from the same specimen, averaging these deviations, and dividing the average by this mean."[11] As shown by his results, the coefficient of variability (unity representing perfect correlation) for basophilic aggregation counts was 8.89 and that for reticulocyte counts was 24.39. In similar manner we found a coefficient for basophilic aggregation counts to be 8.1, while the coefficient for our reticulocyte counts (modified Cunningham procedure) was 7.3.

Correlation of basophilic aggregation and reticulocyte methods

The authors observed in five normal control subjects a range of basophilic aggregation counts between 0.2 per cent and 0.8 per cent (av. 0.56 per cent) which agrees well with McCord's results (range: 0.2–0.9 per cent, av. 0.49 per cent). The reticulocyte range of the same control subjects was found to be from 0.5 to 1.5 per cent (av. 1.02 per cent).

TABLE 3

Reduced counts

DIAGNOSIS	NUMBER OBSERVATIONS	PER CENT RETICULOCYTES	PER CENT BASOPHILIC AGGREGATIONS
Aplastic anemia	3	0	0
Pernicious anemia	5	0.05	0
Carcinoma (pancreas)	2	0.4	0.3
Polycythemia with surgical myxedema	3	0.2	0.18

We found the reticulocyte and basophilic aggregation counts reduced below normal figures simultaneously in cases of aplastic anemia, severe untreated pernicious anemia, malignancy of the pancreas, and in a thyroidectomized polycythemic patient. In the cases of aplastic anemia and severe pernicious anemia where no reticulocytes in 1,000 cells were seen, we could likewise find no basophilic aggregation cell.

Those cases showing elevated counts were: Terminal glomerulonephritis, thrombocytopenic purpura, leukemia with secondary

anemia, pernicious anemia under liver therapy, lead absorption and intoxication, pneumonia, and congenital hemolytic icterus.

In table 5 are gathered a number of patients presenting widely different clinical problems in which the variation in the counts from the normal is not striking. This fact illustrates the absence

TABLE 4

Elevated counts

DIAGNOSIS	NUMBER OBSERVATIONS	PER CENT RETICULOCYTES	PER CENT BASOPHILIC AGGREGATIONS
Glomerulonephritis	12	1.6–3.3	0.9–2.0
Thrombocytopenic purpura	3	2.6–4.3	1.9–2.5
Leukemia	7	3.7–8.2	3.3–7.6
Pernicious anemia under liver therapy	25	1.5–30.7	1.0–24.6
Lead poisoning (rabbit) with Pb acetate	26	3.0–22.8	1.3–21.6
Pneumonia	2	1.2–1.8	0.9–1.6
Congenital hemolytic anemia	1	30.0	23.5

TABLE 5

Normal range

DIAGNOSIS	NUMBER OBSERVATIONS	PER CENT RETICULOCYTES	PER CENT BASOPHILIC AGGREGATIONS
Polycythemia	2	0.8–1.5	0.3–0.4
Infectious mononucleosis	1	0.7	0.3
Pernicious anemia	1	0.6	0.3
Hodgkins disease	1	0.7	0.8
Aplastic anemia	1	0.9–1.1	0.5–0.7
Nephritis	2	0.9–1.4	0.2–1.0
Hypertension	12	0.08–0.5	0.07–0.4
Liver cirrhosis	4	0.8–1.4	0.2–1.04
Pneumonia	3	0.5–1.3	0.2–0.6
Carcinoma (rectum)	2	0.8	0.7

of any relationship between disease entities and erythropoetic activity.

It next seemed of importance to study the response of the bone marrow to various types of stimulation.

1. Liver therapy. Four cases of Pernicious Anemia were studied before and during liver therapy. The reticulocyte and

Chart I

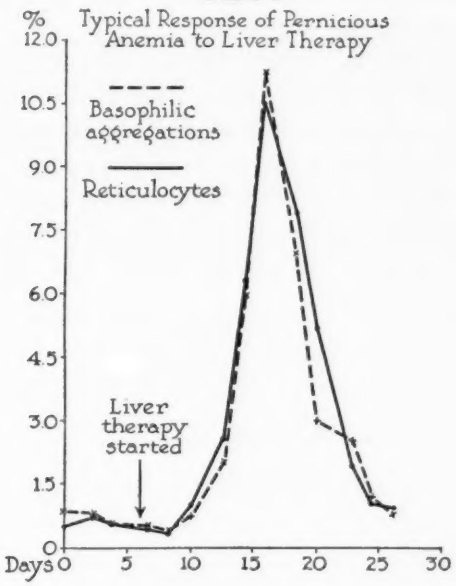
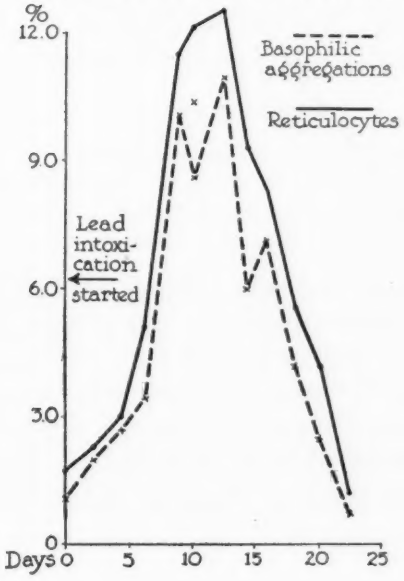


Chart II

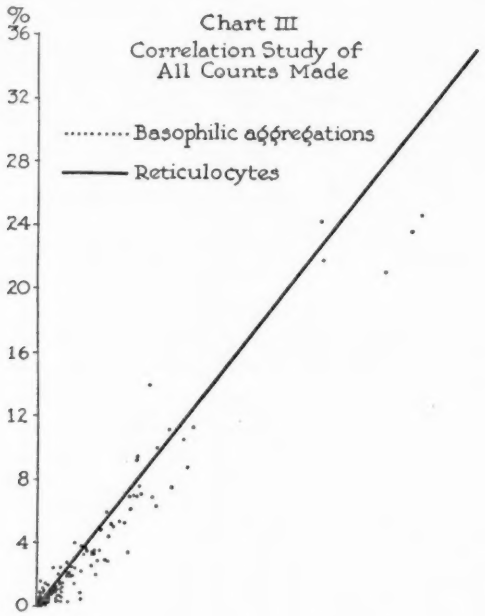
Typical Response of Rabbit's Blood to Intoxication with Lead Acetate



basophilic aggregation responses are shown in chart 1, and it is noted that they are quite similar.

2. Lead intoxication. Again the similiarity in response of the reticulocytes and basophilic aggregation is shown when the blood of rabbits, intoxicated with lead acetate by mouth, is studied.

In chart 3 a correlation study of all the counts made is shown. The reticulocyte counts are shown as a straight line, and the corresponding basophilic aggregation counts are shown as points with reference to this line. It will be noted that the correlation is



good with a high concentration of points immediately about the line. The reticulocyte count was numerically higher in 80 per cent of the cases. In 20 per cent the basophilic aggregation count was higher. Possibly the duration of the staining time, the speed of the reaction, the mechanical treatment of the cells as suggested by Keys and the distribution of basophilic material in the cell may be factors in bringing about a chance distribution of this character.

CONCLUSIONS

1. The Basophilic Aggregation Test is unreliable as a routine laboratory procedure because of errors which have been pointed out in the test.

2. The Basophilic Aggregation Test was applied in a series of 46 patients with widely different clinical diagnoses. It is apparently another method for staining reticulocytes and shows a strong correlation which counts made according to a "vital" staining method.

3. In some of these patients the bone marrow was under-going stimulation, in others it was relatively aplastic, and in still others there was no evidence of stimulation. In 80 per cent of cases the reticulocyte count was somewhat higher then the basophilic aggregation count.

4. The Basophilic Aggregation Test is not uniquely diagnostic of lead absorption or intoxication.[33] It is an index of marrow response. The basophilic aggregations are increased when there is any cause for an erythroid response.

5. The basophilic aggregation test should be abandoned in favor of the "vital" method for staining reticulocytes.

The authors wish to thank Dr. Robert W. Keeton, at whose suggestion the study was undertaken, for help and constructive criticism in completing it.

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THE EBB AND FLOW OF THEORIES ABOUT PERNICIOUS ANEMIA*

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The radiologist and the clinical pathologist are relatively recent additions to the medical hierarchy, and both are forced to act as medical encyclopedias, supplying diagnoses and even outlining treatment for nearly all the ills known to science. Although these specialists view with alarm or with acrid mirth the practitioners whose study of patients includes laboratory or radiological observations, they usually regard themselves and are regarded by their colleagues as men who know everything. They deal with visible evidence of disease, with lesions which can be measured or chemical changes which can be titrated, and they have great confidence in the relative security of diagnoses based on such data, very little respect for the evidence supplied by circumstantial symptoms and signs. It is therefore not without disciplinary value for clinical pathologists to review the history of a disease whose manifestations they study with great assurance, especially as pathologists of wide renown played a large part in the story of this condition. The history of pernicious anemia shows how greatly the experts vary in their interpretation of visible evidence, how easily men narrow their mental vision to the fashionable field, and how often their confident opinion is founded on pure fantasy.

One hundred and sixteen years ago Combe of Edinburgh described the disease which custom decrees shall be labeled pernicious anemia. He noted cases described in 1684 and 1761 by Reiselius and Leiutaud and casually remarked that "it is prob-

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ably owing to some disorder of the digestive and assimilative organs that its characteristic symptom has its origin and to the correction of this derangement we must look for a removal of the disease." Addison's more complete and classical description of the clinical features was published in 1849; it was while studying this anemia that he discovered the disease of the adrenals which bears his name. By 1855 he was willing to hazard a guess that "some form of fatty degeneration might have a share" in the production of the anemia. The sore tongue had been described by Barclay in 1851 and 20 years later the atrophic gastritis was emphasized by Fenwicke and by Austin Flint, who independently reached the conclusion that the disease was a direct result of an imperfect secretion of gastric juice. Ewald and also Martius, the first actually to demonstrate achylia gastrica in this disease, regarded the gastric defect as primary. So did Osler, Pepper, Stengel and others who had observed the gastric atrophy, but most German observers thought the gastric disorders were due to secondary lesions.

Up to 1928, very little progress had been made along this line, although Faber, Hurst, and Levine had emphasized the constancy and the first two had proved the primacy of achylia. This was even more convincingly demonstrated after Minot introduced liver therapy, which corrected the anemia but left the achylia unchanged. While most investigators were concentrating their attention on liver therapy, Castle returned to the study of the part played by gastric defect, and proved that normal gastric juice plus beefsteak will evoke a remission. Only then did it occur to several investigators to try dried pig stomach for pernicious anemia just as dried thyroid had been given forty years earlier for myxedema. The success of this experiment brilliantly confirmed the guesses of the early students, who were sure the disease was due to a gastric defect but did not apply to this deficiency the sort of therapy they knew was effective in thyroid dysfunction. Further work, in Scandinavia, has shown that the ferment-like substance can be obtained from the pylorus, duodenum and entire small bowel of the pig.

Ganslen showed that the liver extract of Minot was very poorly

absorbed if given by mouth, since quantities of extract only one-tenth to one-thirtieth the oral dose were adequate if given by parenteral injections. The substance in the gastric juice and dried mucosa is quite unlike that in liver, but given with liver extract it makes oral therapy much more effective, presumably by increasing assimilation of the essential substance. It seems probable that beef muscle contains the same material as liver, but in quantities too small to be effective unless absorption is increased by gastric or enteric ferment. For some reason, parenteral administration of muscle extract seems not to have been tried, but extract of muscle which had been incubated with gastric juice was potent in Wilkinson's experiments. It is noteworthy that most animals do not have the gastro-enteric ferment, yet even those like cattle which have no dietary source of the curative substance have large quantities of this material in their livers. Man probably can also synthesize the effective substance from a vegetable diet, but in most cases of pernicious anemia the patient suffers, as Combe surmised 107 years before Castle's crucial test, from inability to digest and assimilate something needed to remove the disease. How this "something" is used, what rôle it plays in the maintenance or protection of the blood and nerve cells is still a mystery.

The gastro-intestinal defect theory was long lost to view in a war over the interpretation of the changes in the blood and marrow. Microscopic study of the bone marrow led Pepper, in 1875, to describe the disease as a "pseudo-leukemia," while Cohnheim, the following year, interpreted the hyperplasia as evidence of return to an embryonal state with retention of immature forms as the primary factor in the disease. Neumann, however, regarded the marrow change as secondary and compensatory, similar in nature to the hyperplasia due to hemorrhage. Ehrlich extended and refined the views of Cohnheim, for he observed macrocytes and megaloblasts in the circulating blood, and emphasized the megaloblastic nature of the marrow as evidence of the embryonal reversion. He rejected the leukemic analogy of some theorists, and considered the megaloblastic reversion to be a result of the action of a poison, or of a group of poisons which caused blood destruction and interfered with

blood formation. It should be emphasized that Ehrlich did not regard megaloblastic blood or marrow as evidence of a single specific disorder. One of the cases he included in his study of megaloblasts was a case of generalized sarcomatosis. The megaloblasts were specific evidence that the patient had progressive or pernicious, or progressive pernicious anemia, but not that his trouble necessarily was due to the same process as that of all other cases of this type of anemia. Biermer, who added nothing to our knowledge of the disease, had presented several reports on "progressive pernicious anemia" about 1870. He gave a confused clinical description as a result of mixing together various diseases under one heading. The title "Progressive pernicious anemia" was a smash hit and has echoed down the years, applied now to pure Addisonian anemia, now to easily cured diseases like fish tapeworm anemia, or to all macrocytic hyperchromic anemias, or to all anemias with megaloblasts in the blood or to all anemias cured by liver extract. Ehrlich accepted the idea that there was no clinical or etiological unity to this group, and made megaloblastic and pernicious anemia synonymous.

While Muir and other careful students very early advanced the view that megaloblasts occurred in normal marrow and were numerous in conditions different from pernicious anemia, Ehrlich championed the thesis that such cells occurred only in embryonal life and in this disease, and he held, as do Downey, Piney and Naegeli today, that these cells are members of a developmental series which is distinct from that of the normoblast. Nearly all English and American texts and teachers now speak of megaloblasts as one stage in the evolution of the normoblast, a fact which has drawn from Naegeli a biting description of the decline of medical acumen. Yet it should be remembered that Naegeli admitted the occurrence of large normoblasts or macroblasts indistinguishable from megaloblasts, that he accepted the view that megaloblasts were usually absent in sprue and advanced this as an argument against the anemia of sprue being like that of pernicious anemia, and finally that Naegeli rejected Ehrlich's chief distinguishing landmark in differentiating the two types of cells. For Naegeli considered nuclear extrusion as an artefact and believed both megaloblasts and normoblasts lose their nuclei

by lysis, while Ehrlich felt that the latter lost the nuclei by extrusion, the former by lysis. It is now generally agreed that huge megaloblasts may occur in hemolytic anemias unlike pernicious anemia.

There is some ground for the belief that Ehrlich and his followers placed far too much emphasis on hematological evidence, and clinicians have astonished expert hematologists by evoking with liver extract classical remissions in cases with cells too small and too poor in hemoglobin to warrant a laboratory diagnosis of pernicious anemia. There are cases of hemolytic anemia, cured by splenectomy, which at times showed proper blood and marrow morphology for Addisonian anemia and also cases of Addisonian anemia, clinically obvious enough and responding normally to specific therapy, which lack the classical blood findings of hyperchromia and macrocytosis and whose marrow contains too few cells typically megaloblastic to be decisive. Uncertain as is the interpretation of the alterations in the cytology of blood and marrow, it was the appearance of the marrow which led Cohnheim and later Peabody to accept arrest of maturation as the outstanding functional defect. Whipple suggested that a substance needed for stroma-building was absent in pernicious anemia and the curative substance from liver is spoken of as a hematopoetic principle.

The theory that pernicious anemia is a hemolytic anemia is also very old. Pigmentation of the liver and spleen were first described by Quincke in 1876, and phagocytosis of red-cells in the marrow was noted by Osler in the following year. They and those who confirmed their observations in the next few years made no deductions from these findings, first because they followed Biermer's original thesis that this type of anemia was due to varied causes, second because similar changes occurred in other conditions and they did not realize how marked the quantitative difference was between the siderosis and phagocytosis in this disease and in others. But William Hunter, a Scot who practiced as pathologist and physician in London, had studied the physiology of blood destruction experimentally, and in 1888 advanced a well-articulated theory on pathogenesis. The glossitis, gastric atrophy, icterus, fever and neurologic signs all were emphasized, and

the evidence of unusually active hemolysis was stressed for the first time. Hunter believed that siderosis in marrow and liver was more marked, as compared with that in the spleen, than in other hemolytic anemias. He concluded the disease was due to the action of a toxin derived chiefly from the flora of the gastro-intestinal tract, and occurring in individuals constitutionally predisposed.

Biermer by this time had switched to the concept of a pure unitarian pernicious anemia and even denied that the fish tapeworm anemia was related; Hunter regarded the tapeworm anemia as one form of this intoxication. He studied the bile pigment excretion, made careful analyses of iron content of tissues, and his series of papers and the book "Severest Anemias" established the hemolytic concept of the disease. His studies on the icterus of this type of anemia was rounded out in 1915 by Addis' work on the high rate of bile pigment excretion in the relapse and low rate in remissions, and this has been repeatedly confirmed by observations both on bilirubinemia and pigment excretion as affected by liver therapy. Minot noted that the serum jaundice cleared up before the reticulocyte shower in treated cases. The rate of pigment excretion also falls to normal prior to or during the reticulocyte shower, as was first shown by Farquharson, and Dobriner has found that the coproporphyrin excretion falls somewhat later, both paralleling the pigment excretion following splenectomy in congenital hemolytic jaundice. The marrow changes were interpreted by Hunter, and also by Muir, Askanazy, Grawitz and other very careful students as evidence of rapid regeneration of marrow cells in response to hemolysis; this view was concurred in by Welch, MacCallum and Bunting who had studied the marrow changes in malarial anemia and in experimental anemia due to saponin hemolysis. This explanation of the marrow changes is that given by Askanazy in the Henke-Lubarsch Handbuch, where the maturation arrest theory is vigorously denounced, and it has been confirmed by work of Steele and of Rhoads and Miller on the marrow changes in experimental anemia. Peabody had pointed out that the bilirubinemia, hemoglobinemia and hematinemia found in active cases were only explicable by assuming a hemolytic process, and his quanti-

tative studies on phagocytosis of the red cells in the marrow had shown that only in cirrhosis of the liver is this met with in a degree approaching that seen in active cases of Addisonian anemia. So strong was his influence and the weight of evidence that Minot first suggested that liver supplied a substance needed to protect or to produce blood. Later Peabody studied biopsies before and after remission and accepted the maturation-arrest hypothesis, but purely on evidence which men familiar with the marrow in malaria or after experimental hemolysis would interpret in the opposite sense. Yet this otherwise wholly unsupported theory gained nearly universal acceptance from 1927 to the present time.

When Fairley observed that quinine in malarial anemia caused a reticulocyte crisis exactly equivalent to that produced by liver in equally severe cases of Addisonian anemia, he concluded that malaria interfered with the maturation of the red cells. There is no evidence that malaria arrests the developing cells except by destroying them, and to liken such a phenomenon to Minot's or Whipple's ideas of maturation arrest in Addisonian anemia is rather far-fetched. Today evidence diametrically opposed to the maturation arrest theory is often interpreted as agreeing with it. On the other hand most recent texts on hematology ignore entirely all the features of Addisonian anemia which point to its hemolytic origin, and do not even mention the existence of such a theory. Pathological texts also describe the morbid anatomy purely in terms of arrested maturation. The excellent morphologic studies of Hunter, Muir, Warthin, Askanazy and Peabody are forgotten, the studies of pigment metabolism are ignored, and the unsolved problem of the etiology of pernicious anemia is concealed. Actually we have a cure, thanks to the genius of Minot, but we have not gone far beyond Combe's century-old theory that a defect in digestion and assimilation is the fundamental cause. The conflict, now 50 years old, as to whether the digestive defect leads to hemolysis or to arrest of maturation is undecided. The evidence for maturation arrest is no better than it was in Cohnheim's day, and is no more convincing to Naegeli and Askanazy, two of the most experienced students of marrow pathology, than it was to the leading hematologists

from 1900 to 1925. The hemolytic theory is far more solidly supported today than it was when Peabody accepted the alternative theory. Yet it is not proved, and even if hemolysis and tissue injury prove to be the cause, it remains to be demonstrated whether this is due to lack of substances needed by red cells, neurones and other tissues, or to lack of a substance necessary to protect against a toxin such as was inferred by Hunter, Ehrlich and Naegeli.

Such is the story of the disease down to our time, but there are many amusing side-shows, such as the nature and fate of reticulocytes, the nature of fish tapeworm anemia, and the distribution of curative material in the gastro-intestinal mucosa and liver of various species of animals. Each of these side-shows presents its own mixture of inspired guesses, absurd hypotheses, and unsolved mysteries. But the chief points at issue have now been clearly defined for just half a century, during which the hemolytic theory had a long period of dominance and promises to enter into another, while the maturation arrest theory, in various forms, has filled the scene for two periods of about 15 years each. Neither theory has ever been universally accepted, and a few pathologists have always clung tenaciously to the unpopular side while their colleagues saw in the blood and marrow whatever it was fashionable to see. To the late William Hunter, pathologist and dean of the medical school at Charing Cross Hospital, belongs the credit for the most original, the most comprehensive, and the most forgotten work which has been recorded in the history of Addisonian anemia. Hunter concluded from his studies that the disease should be treated by removal of all septic foci, with special attention to oral sepsis, and that the diet should be low in animal protein, so as to reduce intestinal auto-intoxication. He removed from the diet exactly those items which we know must be given in very large quantities, so that the practical outcome of his labors was very little, although in 1922 he still reported optimistically on therapy. Whipple, on the basis of some careful observations on experimental anemia, wholly different in nature from this disease, and on the basis of incomplete studies of bile pigment metabolism in dogs, concluded that hemolysis was un-

important in this disease and that liver feeding in various types of anemia might be useful. As a result, Minot was led toward the solution of the therapeutic problem which three groups of investigators simultaneously attacked from the standpoint of diet. Whipple's theories on bile pigment metabolism are entirely discredited, the anti-pernicious anemia factor in liver is inert in the experimental anemia he studied, yet his work greatly hastened the discovery of the cure. All this, I think, has a real lesson for the clinical pathologist, since it shows that advice based on correct and painstaking chemical and morphologic studies may be mischievous, and that the solution of a difficult problem may be found while following a false clue. The pathologist and radiologist are under constant pressure to go beyond their evidence, to take a clinical flyer, and it is well for them to remember that the patient's recovery does not prove that their observations or reasoning are correct, and also that therapy based on accurate observation may be harmful when the observation does not include a wide enough field. Some moralists may hope that the study of the history of pernicious anemia will awaken us to consciousness of some of the beams in our own eyes, and teach us how innocently and easily our colleagues have acquired the occasional mote which seems to us to blur their vision. But the historians now emphasize a fact, strikingly illustrated by the life of the historian and history-maker Woodrow Wilson, that the study of the history of mankind teaches us that mankind learns nothing from the study of history. We may safely conclude that the study of episodes in medical history teaches us how unusual it is for the physician to be saved from falling into pitfalls which the history of medicine should have revealed to him.

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SOME ETIOLOGICAL FACTORS AND LESIONS IN CEREBRAL ANOXIA*

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Asphyxia has been given much consideration by physiologists, but the pathologist has until recently thought of this condition as an acute and perhaps fatal incident associated with strangulation or drowning. It is the purpose of this communication to study, not the obvious types of oxygen want mentioned above, but rather the milder but more prolonged types which invariably result in demonstrable cellular damage. Consideration is given to the anoxia associated with shock, sedation, and fever therapy.

Since sedation is usually necessary in artificial fever therapy, since shock frequently complicates it, and since anoxia of some degree results from it, tissue changes produced by this combination are presented first.

In earlier publications,[1] changes noted in three human cases and some fifty dogs were reported after exposure to artificial fever, in periods ranging from three to eight hours, and temperatures ranging from 104 to 109°F. These changes briefly consisted of marked dilatation and engorgement of blood vessels, degeneration and hemorrhage in the adrenals, brain, liver, lungs and kidneys. Microscopic examination showed acute congestion of the tissues, cellular degeneration associated with hemorrhages in adrenals, brain, liver, lungs and kidneys. When the first description of these pathological changes was presented, their exact etiology and pathogenesis were not appreciated except that they were directly related to the length and height of the fever produced, and that the more severe lesions were most common in

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the experimental animals receiving certain of the barbituric acid group of sedatives.

Comparable changes have been described in heat stroke and in association with burns of the body surface; experimentally by Hall and Wakefield[1a] in the study of heat stroke; by Jacobsen and Hosoi[2] following the application of radiothermy; and by Baldwin and Nelson,[3] Baldwin and Dondale,[4] and Schereschewsky[5, 6] following exposure to high frequency currents. Similar changes have also been described in the brain resulting from entirely different types of experimental and clinical experience. These latter were produced through the medium of anoxia obtained either by ligation of blood vessels typified in the experiments of Gildea and Cobb,[7] or the clinical reports of Courville[8] in his monograph "Asphyxia as a Consequence of Nitrous Oxide Anesthesia."

As noted in a previous communication,[9] the similarity of these lesions found in the brain led to the conclusion that the pathology resulting from fever therapy was also a manifestation of anoxia. In accordance with this conclusion observations on the oxygen content of arterial and venous blood before and after the application of artificial fever were made (tables 1 and 2). These tables show that the normal oxygen saturation of the arterial blood when drawn directly from the femoral artery ranges from 86.5 to 98 and further that animals almost invariably have this oxygen saturation reduced if the fever is maintained over the usual therapeutic period of four to five hours and when the temperature reaches the therapeutic range of 104 to 107°F. It is also noted that those animals which have their oxygen saturation depressed as low as 63 or under succumb. In addition, if the pathology found in the various animals is correlated with the oxygen saturation, the severity of the lesion parallels the depression of the oxygen saturation.

Histological examination of various regions of the brain shows lesions which may be roughly grouped into early and late lesions. The early changes consist largely of edema, noted particularly for the wide perivascular spaces. This observation is supported by the experimental work of Landis[10] who showed that fluid

TABLE 1

Animals receiving fever therapy

DATE 1937	NUMBER	DURATION TREATMENT	TEMPERATURE		ARTERIAL BLOOD			VEN. BLOOD	REMARKS
			Beginning	end	O_2 content	O_2 Capacity	Per cent Saturation	O_2 content	
		hours	*°F.*	*°F.*	*vol. per cent*	*vol. per cent*		*vol. per cent*	
5/11	3	4½	102.2	108	10.84	17.95	60		Died 5:00 p.m.
5/13		4	100.4	108	12.35	20.14	61		Died 5:00 p.m.
5/13	4	4	101.2	107.4	12.18	14.86	82		
5/13	5	4	100.6	108	10.31	17.14	60		Died same day
5/15	4	5	102.2	107	20.90	25.14	83.5		
5/15	4	5	102	107	16.65	22.93	72.5		
5/17	4	5	102	105.2	18.30	21.06	86.6	14.8	
5/17	8	4½	101.4	108	13.07	18.67	70	9.83	
5/18	9	5	102	106	14.42	22.98	63	11.47	Blood sugar 85; died during night
5/18	10	4	101	105.8	19.12	24.44	78	15.53	Blood sugar 66
5/22	9	5	101.8	107.6	12.68	19.10	66.5		
5/22	10	5	100	106	19.87	25.8	77	15.07	
5/25	12	5	101	107	15.81	21.39	72	11.11	
5/25	11	5	102.4	106.6	15.95	26.65	59	11.00	Died following morning

TABLE 2

Normal animals

DATE 1937	NUMBER	ARTERIAL BLOOD			VENOUS BLOOD	REMARKS
		O_2 content	O_2 Capacity	Per cent Saturation	O_2 content	
		vol. per cent	*vol. per cent*		*vol. per cent*	
5/19	1	21.03	24.4	87	18.4	
5/20	2	20.67	24.14	86	14.8	
5/21	3	20.52	23.86	86.5	17.5	
5/21	4	21.71	25.05	86.6	13.84	
5/27*		22.10	24.79	89	17.62	

* Animal receiving 15 grains sodium amytal.

passes through the walls of the capillaries at four times the normal rate after complete oxygen want lasting three minutes. The edema mentioned is particularly marked about the pyramidal

cells of the cortex, the ganglion cells of the base, and the Purkinje cells of the cerebellum. The cell itself in these early stages is frequently shrunken, pyknotic, and poorly stained. The destruction may be evident in few or many of the cells within a given area or areas. The capillary walls may be permeable not only to blood plasma but also to the red cells so that cuffs of hemorrhage may be seen especially in the base about these minute vessels (Plate 1). In the later stages the most characteristic finding is the devastation areas described by Gildea and Cobb.[7] These may be found in any area but have been noted most frequently at the base of the brain in both our experimental animals and human cases. They consist of areas of necrosis varying in size which in the microscopic preparation appear as poorly staining tissue with pyknotic nuclei interspersed with clear zones. The margins of these clear zones are ragged and irregular, hence should not be mistaken for defects produced by the microtome knife (Plate 2).

The evidence presented thus far indicates that anoxia usually occurs in the course of fever therapy and that definite tissue damage results from the more severe anoxias thus produced. To be in a position to prevent severe anoxia and tissue damage during artificial fever the factors or factor contributing to anoxia must first be determined. These contributing factors may be considered under the four types of anoxia: the anoxic, the anemic, the stagnant, and the histotoxic.

The studies of Bischoff, Long and Hill,[11] using short radio waves showed that alkalosis occurred soon after pyretotherapy was begun with a blood pH averaging .76 and a CO_2 combining power of the plasma of 40 volumes per cent. This constitutes one of the factors in the anoxia because slightly alkaline hemoglobin gives up its oxygen to the tissues less readily than normal. In the early stages of artificial fever the CO_2 is blown off producing the alkalosis and at the same time depriving the respiratory center of its usual stimulant, thus allowing rapid shallow breathing. The relatively large amount of dead space in the nose, throat and trachea under these conditions reduces the quantity of fresh air reaching the lung alveoli and in turn

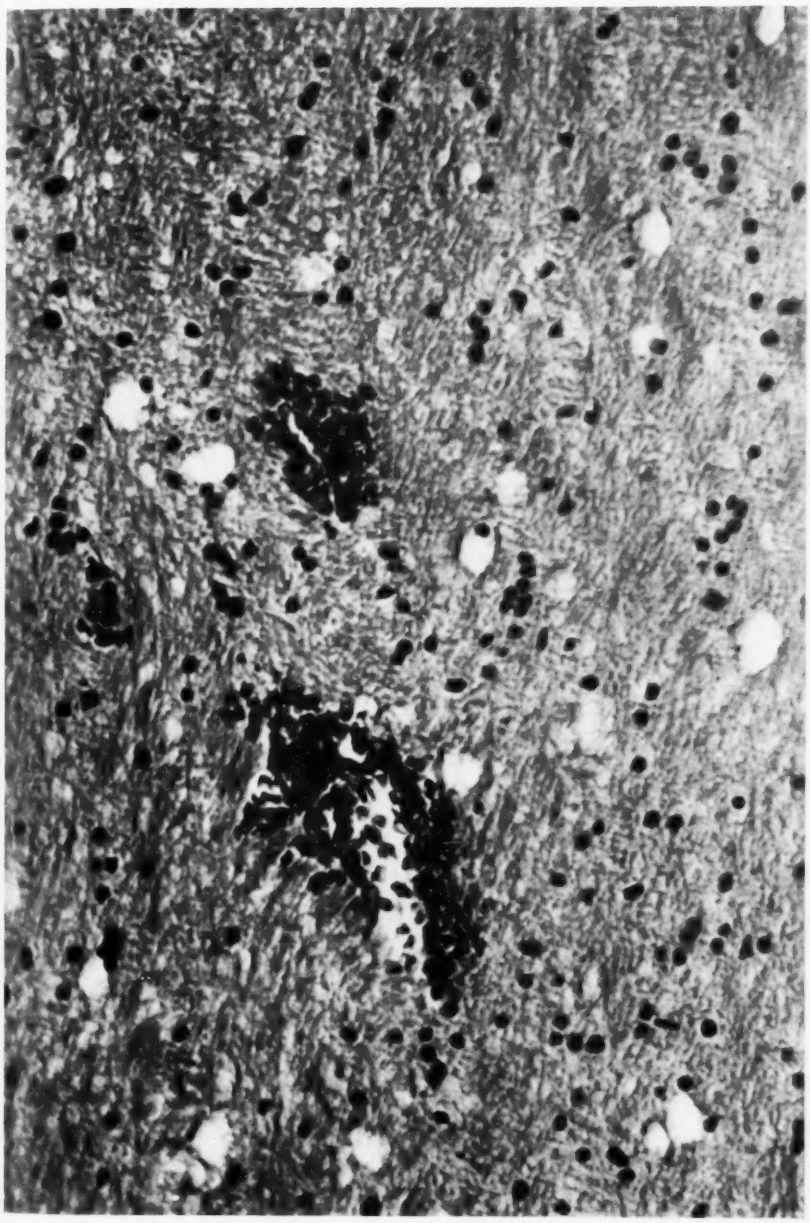

PLATE 1

Photomicrograph medium power. Base of brain (human) showing cuff hemorrhage about small blood vessels.

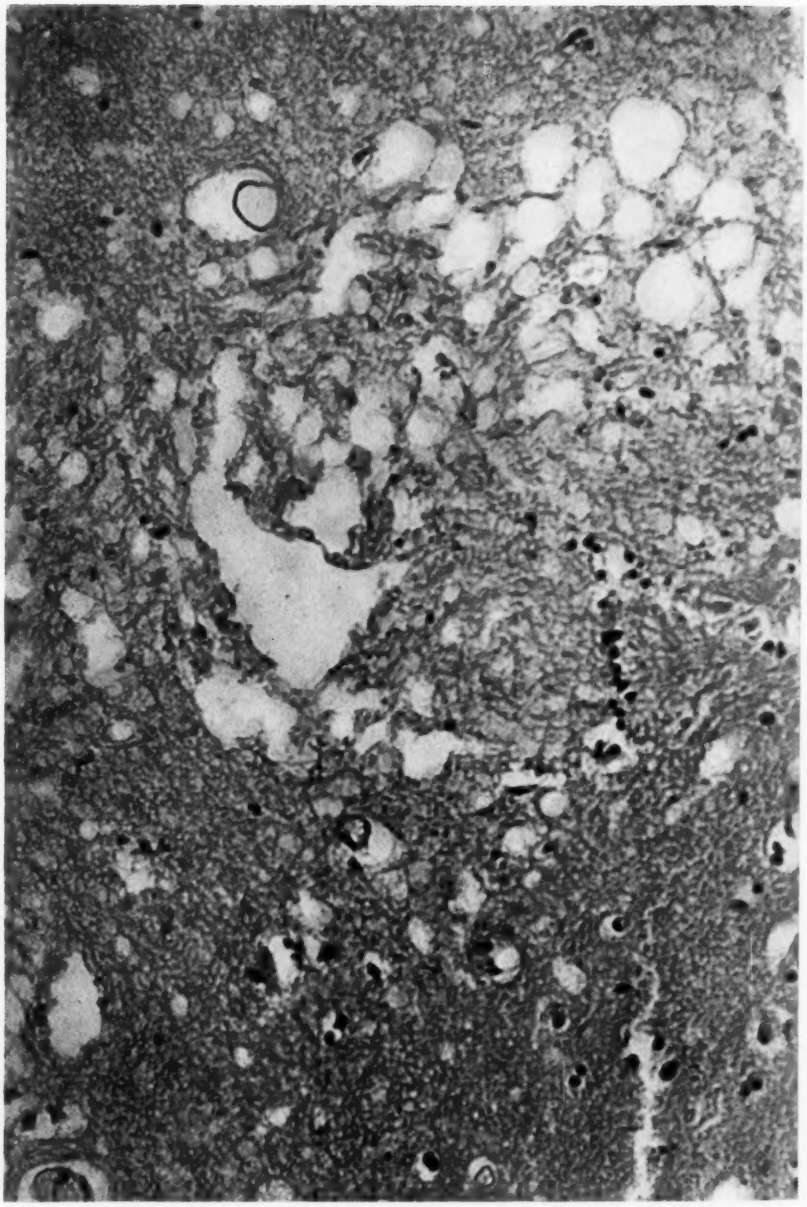

PLATE 2

Photomicrograph low power. Base of brain (human) showing area of "devastation necrosis." Central area shows the tissue necrotic with only a few pyknotic nuclei and large irregular spaces from which tissue has been lost.

the oxygen reaching the blood. Other factors brought into play are the higher temperature of the blood which, as Barcroft[12] has shown, reduces the oxygen saturation and the increased velocity of blood flow through the capillaries demonstrated in artificial fever by Kissin and Bierman[13], Tenney[14], and Bazett[15] which results in a smaller amount of oxygen being removed by the tissues in accordance with the observations of Meakins and Davies[16]. On the other hand, if the cardiac function fails, stagnation takes place in the capillaries and a deficit of oxygen in the tissue follows.

Throughout our experience in fever therapy, it has been apparent that the type of sedative used had a direct bearing on the number of cases developing cyanosis, respiratory and vascular collapse. These complications were attributed to the direct effect of the sedative upon the medullary centers. However, Keilin[17] has shown experimentally that cyanide, alcohol, acetone, and ethyl urethan tend to produce a stable compound of oxycytochrome in the tissues, and hence the oxygen is not readily removed. Recently M. Jowett and J. H. Quastel[18] have shown that luminol, chloretone, and evipal decrease or abolish oxygen utilization by the brain. All of these observations point to an histotoxic factor in the production of anoxia during the fever therapy, hence it is logical to assume that sedatives used in fever therapy not only operate directly upon the respiratory center but more particularly upon the individual cell itself, interfering with the normal cellular respiration.

A review of the literature shows that one of the early studies on the histopathology of the central nervous system during experimental poisoning with medinal was that of T. Nakamura[19] who showed that in the cerebral cortex there was acute Nissl's degeneration; that in the nuclear areas of the mesocephalon the ganglion cells are often swollen with the tigroid changed into fine granules, clumps or homogeneous masses; that in the gliacells of the Hortiga type especially there are pronounced regressive changes; that in the Purkinje cells of the cerebellum there may be severe vacuolar degeneration and enlargement of the axon; and that in the spinal cord there is more degeneration of the ganglion cells than in the medulla oblongata.

Our studies in the dog, using sodium amytal, and on two human cases using medinal with suicidal intent, show essentially the changes noted in Nakamura's work (Plates 3, 4, & 5). Both parallel closely the findings of Courville[8] and others referred to previously.

The use of respiratory depressants, that is narcotics and hypnotics, in labor were reviewed by Henderson[20] in 1937. First, he points out that the baby is more strongly affected than the mother so that dosages that scarcely depress the respiration of the mother may render the infant apneic. In the report of the Chicago Board of Health morphine, scopolamine and various barbituric acid compounds were employed during labor in 79 cases. In 41 of these 79 cases, or 51.9 per cent, the use of these drugs was considered questionable and narcosis of the newborn child resulted. Second, Henderson shows that while CO_2 may be used successfully to combat morphine narcosis it is of little value in barbiturate narcosis, because in such states the normal respiratory stimulant is so nearly abolished that breathing continues principally because of anoxia.

Eastman[21] emphasizes the danger of nitrous oxide in obstetrics by showing that when it is given in concentrations of 10 per cent or more for periods longer than five minutes marked degrees of fetal anoxia are produced in about one baby out of three. Irving[22] shows that only 1.9 per cent of infants do not breathe immediately after birth when the mother is unanesthetized while 20 per cent do not breathe immediately when the mother is anesthetized. Schreiber and Gates[23] have called attention to cerebral damage resulting in infants when a combination of narcotics and nitrous oxide anesthesia is used during labor. These warnings are impressive when it is found that in a small series of obstetrical patients under conservative doses of nembutal and light nitrous oxide anesthesia during delivery the oxygen content of the arterial blood is reduced from 5 to 15 per cent. Plates 6 and 7 illustrate brain lesions found in this type of anoxia from the child of a 29-year-old multipara. The mother was given 3 grams of nembutal and 1/150 of scopolamine during the first stage of labor. During

PLATE 3

Photomicrograph medium power. Base of brain (dog) showing group of ganglion cells stained with cresyl violet for Nissl bodies. Shows marked vacuolation and degeneration with clumping of the Nissl material.

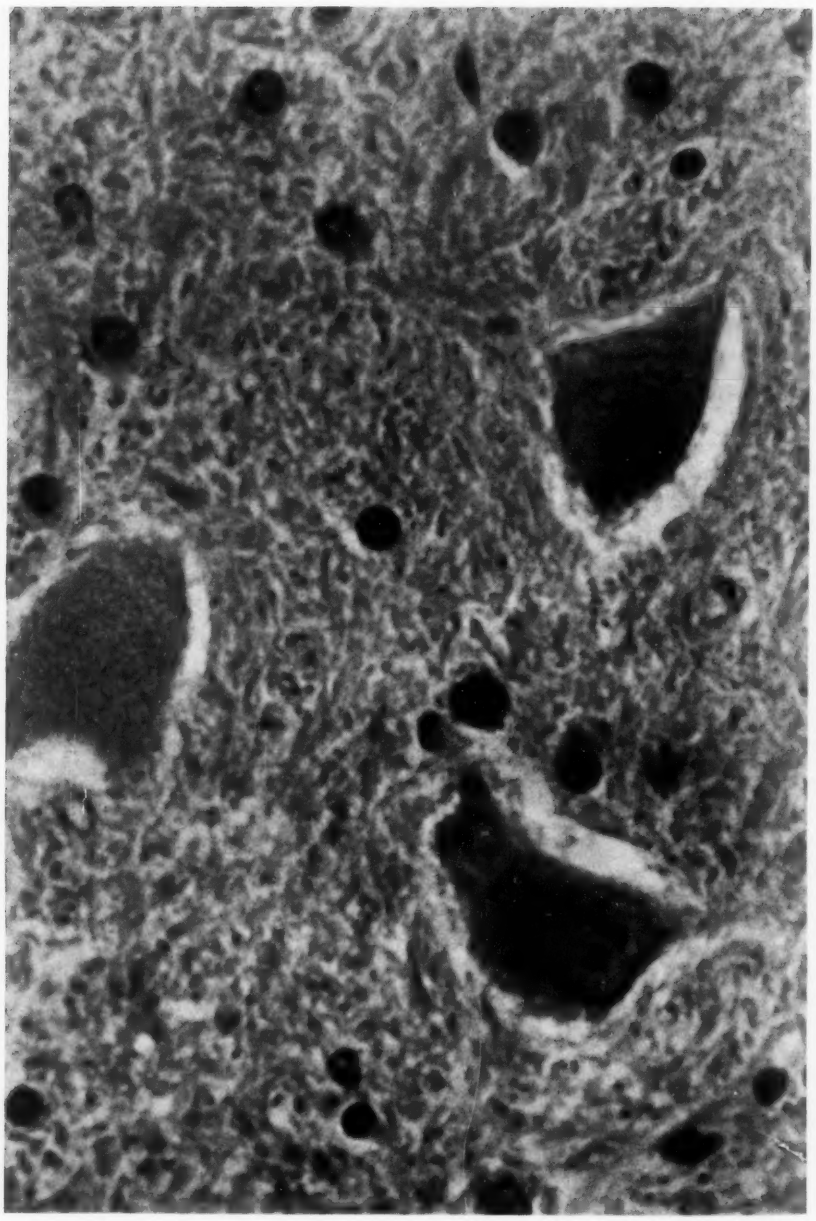

PLATE 4

Photomicrograph medium power. Base of brain (human) showing ganglion cells with hematoxylin and eosin stain. Marked pericellular edema is noted with granular degeneration of the Nissl material.

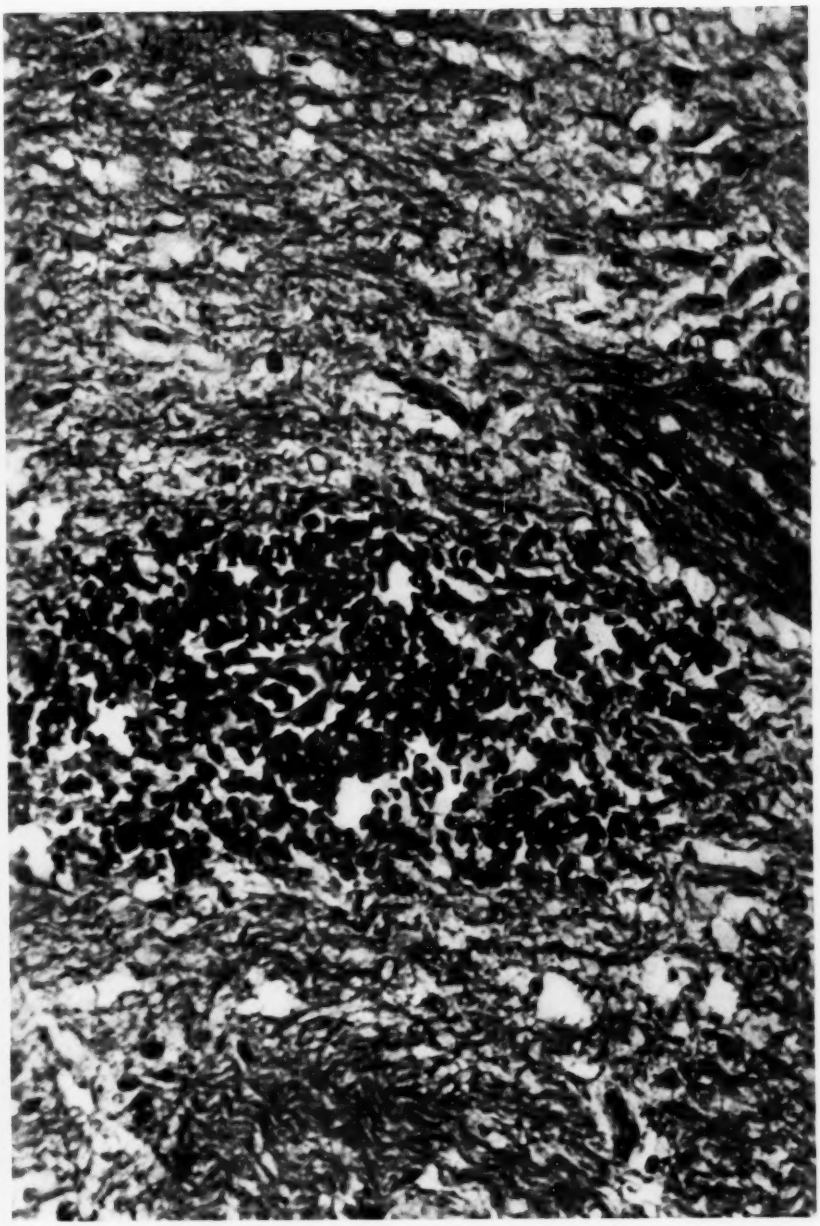

PLATE 5

Photomicrograph low power. Base of brain (human) showing diffuse hemorrhage about small blood vessels. Also marked pericellular edema and vacuolar degeneration of the surrounding tissue.

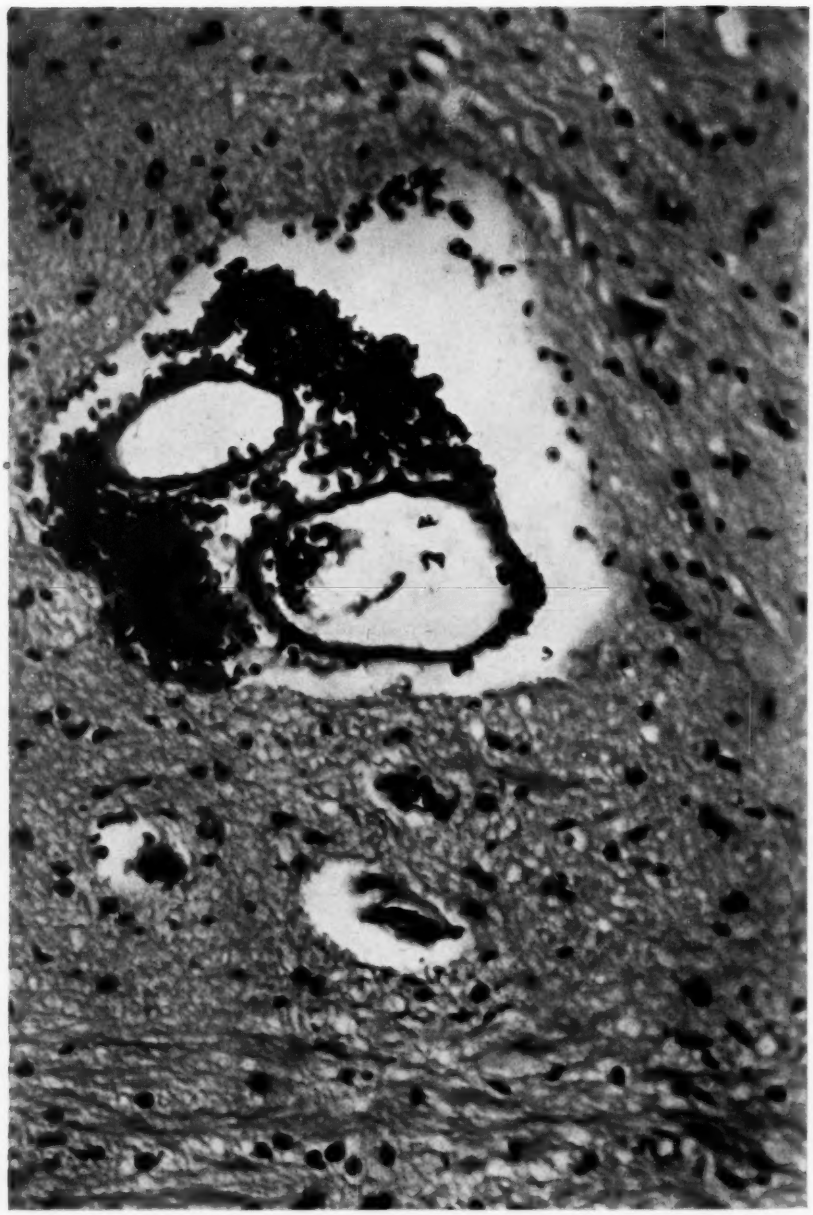

PLATE 6

Photomicrograph low power. Base of brain (human infant) showing cuff hemorrhage about small blood vessels.

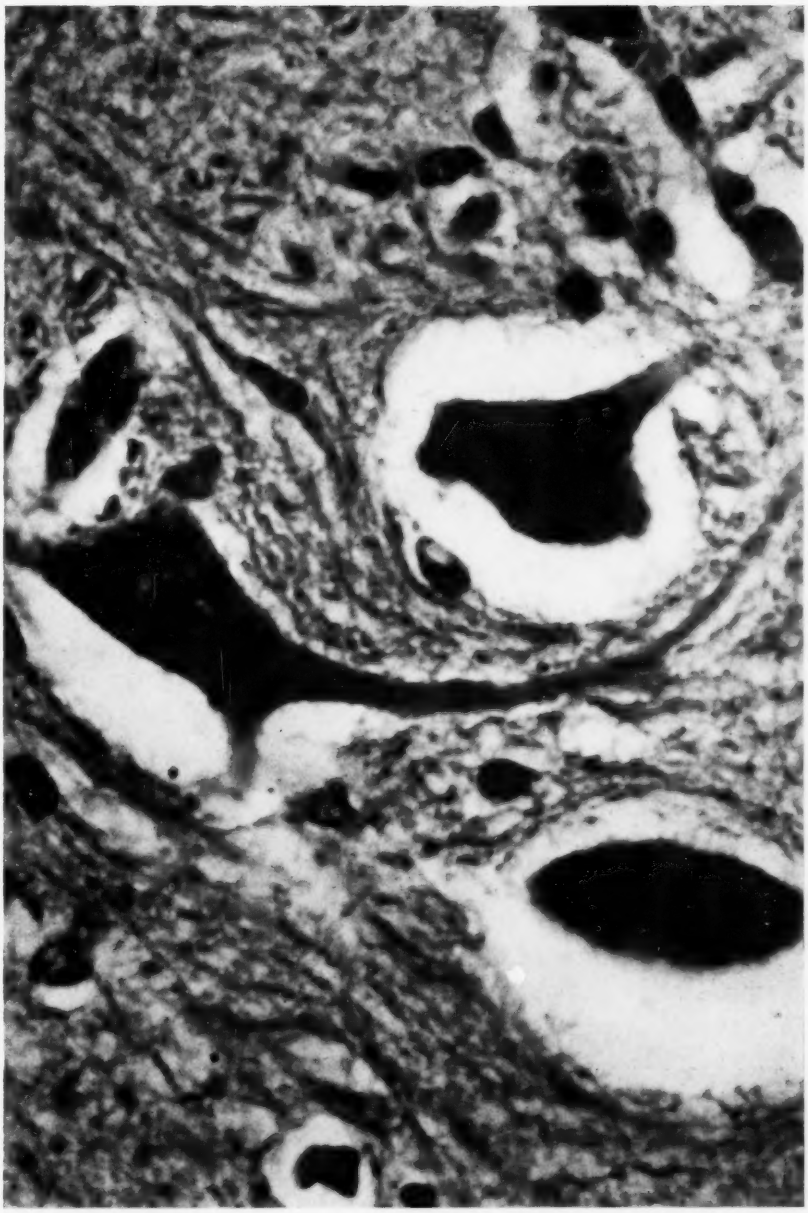

PLATE 7

Photomicrograph medium power. Base of brain (human infant) showing marked pericellular edema with shrinkage and pyknosis of two of the three ganglion cells shown.

the second stage of labor nitrous oxide oxygen inhalations were administered. The child's color was not good at delivery and it was given coramin and CO_2 + O_2 inhalations. The child continued cyanotic, dyspneic and limp. Respirations were shallow and rapid. Despite therapeutic measures the cyanosis deepened and respirations failed about 18 hours after delivery. Autopsy showed no gross cerebral hemorrhage but marked engorgement of vessels.

The work of Keilin[17] referred to above, shows that among other drugs alcohol is one which tends to produce a stable compound of the respiratory pigment oxycytochrome in the cells. Because of this action alcohol tends to produce the histotoxic type of anoxia and in acute alcoholism sufficient vascular collapse and shock may be present to produce in addition the stagnant type of anoxia. Two cases of acute alcoholism with lesions of cerebral anoxia have been examined in the last year, one through the courtesy of the coroner's office and the other through Dr. G. Steiner, Neuropathologist at Wayne University College of Medicine. Large but unmeasured amounts of alcoholic beverages were imbibed in each case. The first case also received three $\frac{1}{4}$ grain doses of morphine to counteract the stimulating effect of the alcohol. After the last morphine the patient lapsed into coma, accompanied by slow respiration and marked cyanosis. In this state he was sent to the hospital. Artificial respiration intermittently and other measures kept him alive only twelve hours. During all of this time the systolic blood pressure was below 68 and the diastolic frequently could not be obtained. Pulse ranged from 108 to 116. Partial autopsy showed the blood unclotted. The lungs were increased in density with marked edema and congestion. The brain showed marked engorgement of vessels with small areas of hemorrhage. Microscopically there is marked pericellular and perivascular edema with small extravasations of blood in blocks from the base. There is marked degeneration, especially of the ganglion cells, as evidenced by the granulation and clumping of the Nissl bodies (Plates 8 and 9).

The fifth and last group of cases showing almost identical

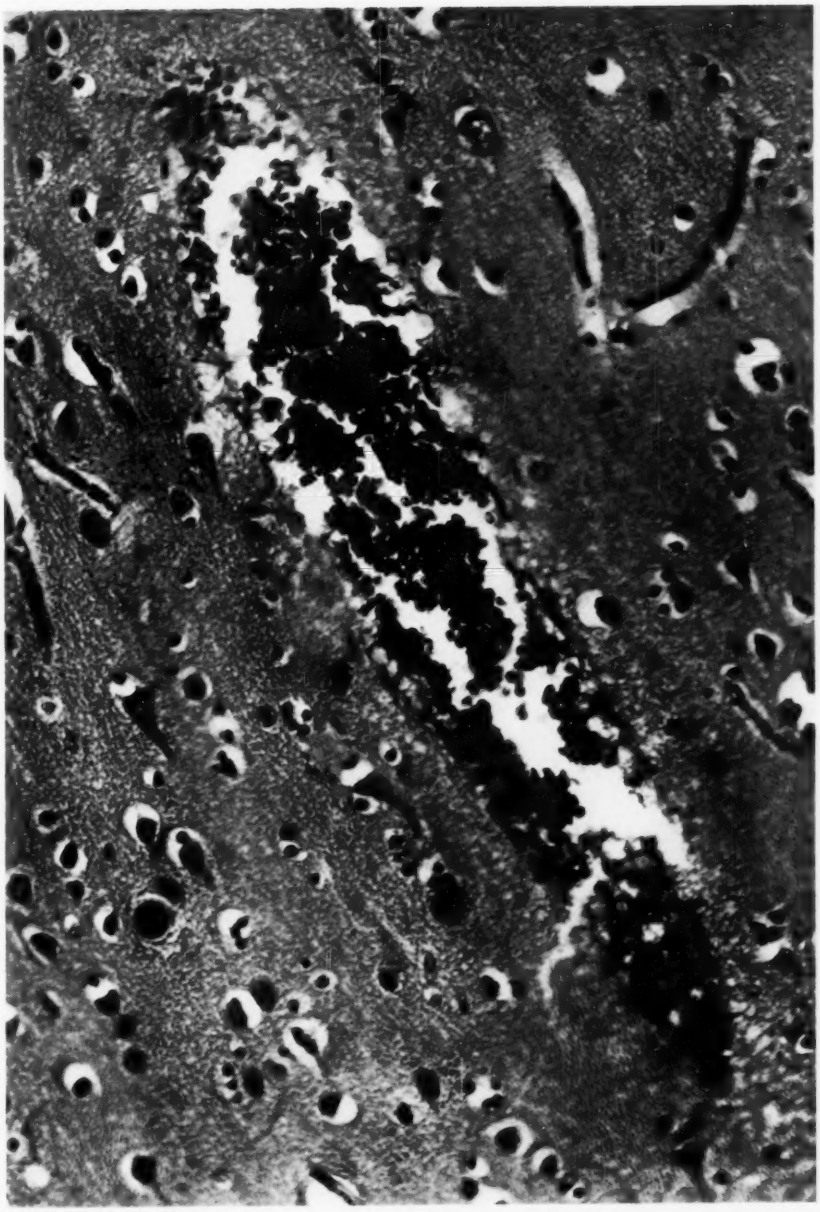

PLATE 8

Photomicrograph low power. Cortex (human) showing large perivascular hemorrhage and pericellular edema.

PLATE 9

Photomicrograph low power. Base of brain (human) showing small vessels with perivascular hemorrhage.

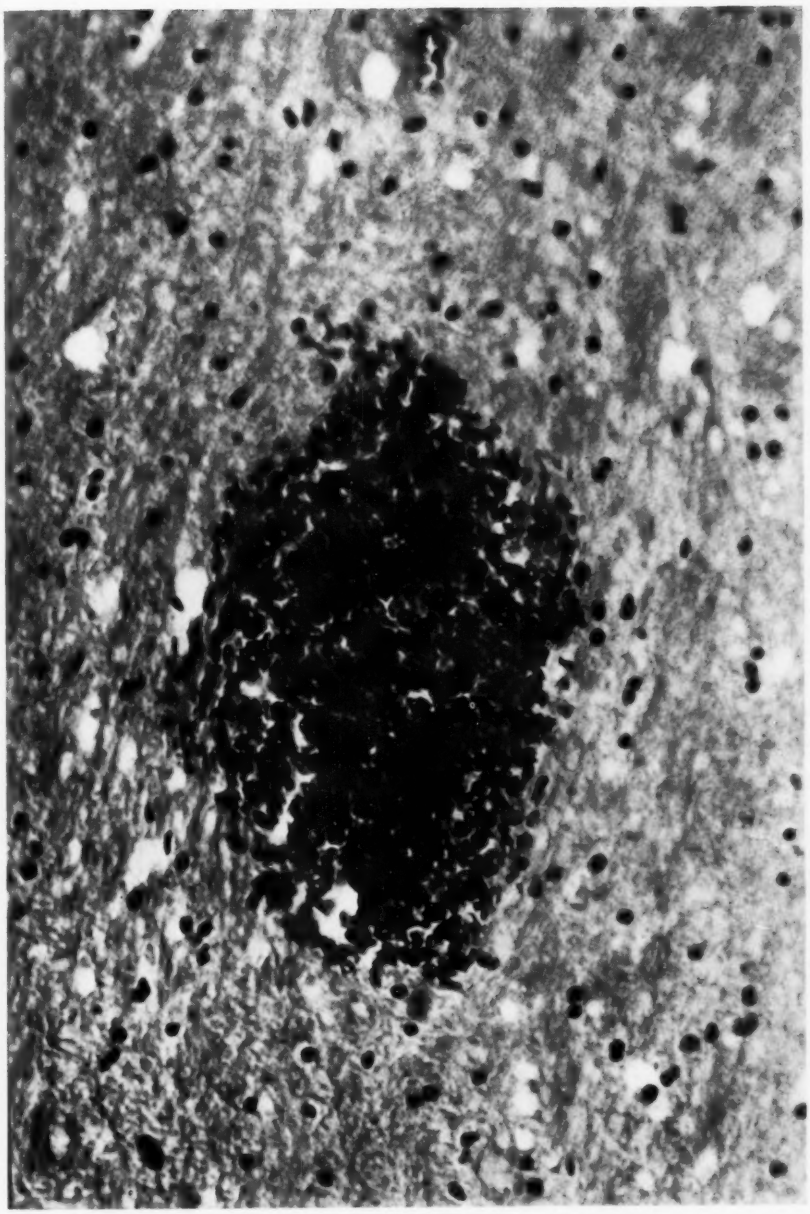

PLATE 10

Photomicrograph low power. Base of brain (human) showing cuff hemorrhage.

PLATE 11

Photomicrograph low power. Base of brain (human) showing margin of large area of devastation necrosis. Peripheral tissues well preserved. Nuclei stain normal in the lighter staining area. Nuclei pyknotic and there are large areas from which the tissue has been lost.

pathological changes is the so-called concussion group. In these the exact mechanism is still unsettled but probably it involves, as suggested by Hoff[24], Gildea and Cobb[7], Winkelman and Eckel[25] and Schaller, Takami and Newman[26] and others, edema, vasodilatation, stasis and anoxemia which in turn result in ring hemorrhages, Nissl degeneration, especially in the ganglion cells, and areas of devastation necrosis varying in size. A good example is offered by a colored patient of 45, admitted after having been struck by an automobile. He was unconscious for a short time, then complained of chilliness and generalized tremor. Examination showed brush burn on the side of the face but the skull was not fractured. There were fractures of several ribs, and tibia and fibula on the right. On the second day the patient lapsed into coma, showing rigidity of neck and spastic extremities. The clinical impression was cerebral laceration and hemorrhage. Coma continued and constant nystagmus was noted on the third day. The patient expired about seventy-eight hours after admission. Autopsy revealed no gross laceration and no meningeal hemorrhage. There was marked engorgement of the vessels and edema. Sections showed petechial hemorrhages throughout the white matter of the cerebrum and the mesencephalon. Microscopically in addition to the hemorrhages comparatively large areas of devastation necrosis are found (Plates 10 and 11).

DISCUSSION

In the five groups of cases presented the pathological lesions are similar, both grossly and microscopically, and it seems certain that anoxia is the underlying and most important etiological factor in their production. The exact mechanism of the anoxia differs somewhat in these groups and all have more than one physiological type operating. In barbiturate poisoning we have early, nearly pure histotoxic anoxia, but later the stagnant type comes into play through the low blood pressure. In acute alcoholism there is again histotoxic anoxia plus the stagnant type and in one case described anoxic anoxia resulting from morphine administration and respiratory failure. In the newborn histotoxic anoxia results from drugging of the mother and anoxic

anoxia if the delivery is done under nitrous oxide anesthesia. In concussion there is stagnant anoxia due to edema and vasodilation with possible histotoxic anoxia as a direct effect of the blow. Shock with falling blood pressure may also be a factor. In fever therapy cerebral anoxia is due to a combination of histotoxic anoxia resulting from heavy sedation in the face of the high temperatures which increase the oxygen demands of cellular metabolism. Stagnant anoxia may also play a rôle if vascular collapse occurs.

SUMMARY

The lesions of cerebral anoxia are rather uniform when all the numerous factors operating to produce them and the wide range of variation due to time of examination in relation to the injury are considered.

Minute gross and microscopic examination of all parts of the brain with the aid of special stains are necessary.

More extended determination of the oxygen content of the arterial blood is urged where anoxia may be a factor. Unfortunately arterial blood tells little regarding cellular anoxia and simplification of present methods for estimating tissue respiration or development of new methods for this purpose are paramount if these studies are to be extended and are to reach the place of clinical importance they deserve.

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NEWS AND NOTICES

Tenth Annual Report of the Board of Registry of the American Society of Clinical Pathologists for the Period May 1, 1937, to April 30, 1938

To the Officers and Fellows of the American Society of Clinical Pathologists:

Ten years have elapsed since our Society established the Registry of Medical Technologists. It is but fitting therefore that we take a retrospect of its accomplishments during the first decade of its existence. It was a pioneering step that the American Society of Clinical Pathologists took in its endeavor to make order out of the chaos prevailing ten years ago when no regulations whatever existed for the appraisal of the qualifications of laboratory technicians. The training of these workers had been more or less haphazard and inadequate; their preliminary education more or less scanty. In this untilled soil the Board of Registry, feeling its way, gradually but steadily introduced norms and standards which in the course of years become more and more elevated, always aiming at the highest possible level of scientific and cultural attainment with due regard to the usefulness of the Medical Technologist to the Clinical Pathologist, the physician, the hospital, and, of course, the central object of interest, the patient. Thus, in the early years but a high school diploma, or equivalent, was a requisite, and with but six months of practical experience and a mere recommendation from a Clinical Pathologist. In 1933, formal examinations were instituted and one year of a college course including chemistry and biology, with twelve months of practical instruction, were made obligatory. Now since the beginning of 1938 two years of college, with a definite pre-training curriculum in the basic sciences, are required before entrance into the vocational course.

All this advance could not have been accomplished without some creaking of the machinery. Appeals for exemptions and exceptions had to be tactfully but firmly resisted in order to reach the goal in view. Speaking of machinery, the growth in the number of registrants, the enormous correspondence involved with applicants, Clinical Pathologists, hospitals and training schools necessitated a corresponding increase in the official armamentarium and personnel, assuming the magnitude of a large business organization. This may be illustrated by one table (see p. 652) showing the number of new and total registrants for each of the ten years.

Seven successive editions have appeared of a booklet giving detailed information of the work of the Registry and the standards laid down for registrants as well as for training schools. Similarly, annual printings have been made of the Directory of Registered Medical Technologists, arranged alphabetically as well as geographically. Copies of these are sent gratis to every registrant and to

YEAR	NEW REGISTRANTS	DROPPED	REINSTATED	TOTAL
1929–30	469			469
1930–31	323			792
1931–32	271	8		1055
1932–33	894			1949
1933–34	175			2124
1934–35	296			2420
1935–36	783	30		3164
1936–37	636	33	8	3775
1937–38	831	143	2	4465

every Fellow of the Society. Through the counsellors, the Registry is apprised of any infraction of the Code of Ethics by a registrant, and no renewal of the certificate will be issued.

The high repute in which the Registry is held as the quasi-official qualifying body for Medical Technologists of the United States and Canada is attested by the imposing number of close to 5000 registrants (including the recent successful candidates) on the roster. The Council on Medical Education and Hospitals of the American Medical Association and the American College of Surgeons, through their field inspectors in their visits to hospitals, make it a routine to ascertain whether the technicians in the laboratory carry a certificate from the Registry. This has greatly stimulated the number seeking registration and brought about a more scientifically trained personnel in the hospital laboratories.

Our aim is also to educate the general practitioner to the danger of employing inferior technicians and to either refer the work to a Clinical Pathologist or, if his practice warrants, to hire only registered help. Besides the danger of faulty findings leading to errors in diagnosis, the unqualified technician is an economic menace to those who have spent the necessary time in proper training.

Gone are the days when the Clinical Pathologist resented the advent of the Medical Technologist. In the present era of large scale routine laboratory tests both in the hospital and at the office, the Medical Technologist has become as indispensable as the nurse to the doctor, and the Clinical Pathologist may save his valuable time to interpreting and evaluating the findings in his rôle as a director and consultant.

In order to keep the work of the Registry before the profession, a traveling exhibit, designed by Doctor Roy R. Kracke, has made the rounds of various medical conventions, such as the

American Medical Association—Atlantic City
Catholic Hospital Association—Chicago
American Hospital Association—Atlantic City
Texas State Society of Medical Technologists—Dallas
Oklahoma State Medical Society—Oklahoma City
Texas State Medical Society—Galveston.

An additional task assumed by the Registry from its inception is the supervision of the training of Medical Technologists, laying down standards for their preliminary education as well as the duration and character of the instruction. Gradually the requirements were raised from a high school diploma to a minimum of two years in college, with emphasis on the basic sciences of chemistry and biology. Universities have been encouraged to give a four year course leading to degrees of Bachelor of Science in Medical Technology, the last twelve months to be spent in a proper hospital laboratory under the supervision of a Clinical Pathologist.

The activities of the commercial schools, organized for gain and offering but poor instruction, have been greatly curtailed, and their "racket" will die with the wider publicity that is now given to the work of the Registry. The Council on Medical Education and Hospitals of the American Medical Association has been very helpful in the approval of schools by sending their field men to inspect the institutions and by issuing a list similar to that of the Registry. Steps are now being taken to publish an identical list approved by both bodies.

The following data for 1938 will be of interest:

		Enrollment
Approved schools:		
College courses	22	749
Hospital Schools	123	577
Total	145	1,326

A Model Curriculum, the work of Doctor I. Davidsohn of our Board, has been distributed to the schools and their students at cost to insure thoroughness of instruction and a certain degree of uniformity in teaching. Many registrants have sent for the book to aid them in review and post-graduate instruction.

To keep Medical Technologists abreast of the times, the Registry is now engaged in a survey of the facilities of universities and Boards of Health for giving short intensive courses in the various fields of clinical pathology. Auspicious beginnings have already been made in a number of localities.

A financial report, audited by a certified public accountant, of the income and expenditures of the Registry has been submitted to the Executive Committee. The satisfactory state of the finances is due to the labor of love on the part of the members of the Board, who serve without compensation, and to the voluntary cooperation freely given by the 130 examiners and their aides who leave their regular duties to spend a day in examining our applicants, even contributing the cost of animals and materials.

The expense of carrying on the work of the Registry is borne almost entirely by the $10.00 examination fee. The nominal renewal change of One Dollar barely covers the cost of the certificate, roster, and accompanying correspondence.

As a result of the ten years of activity of the Registry as an integral part of the American Society of Clinical Pathologists, we may, in all modesty, submit

that it has greatly enhanced the prestige of our organization in the medical and hospital world. Every certificate that bears our cachet is a potent reminder of the important rôle that the Clinical Pathologist plays in the scientific practice of medicine.

The success achieved in the first decade of enrolling close to half of the laboratory technicians of the country gives hope of gathering all qualified Medical Technologists under our banner in the near future.

PHILIP HILLKOWITZ, M.D., *Chairman*
KANO IKEDA, M.D., *Secretary*
ISRAEL DAVIDSOHN, M.D.
H. H. FOSKETT, M.D.
ROY R. KRACKE, M.D.
ASHER YAGUDA, M.D.

Announcement is made that the rental fee for the tumor sets available from the Tumor Registry has been reduced to $2 for the first week and $1 for each week thereafter.

OBITUARY

PHILIP B. MATZ, M.D. was born in Baltimore in 1885 and received his early education in that City and New York. A graduate of Mather College (Lit. B.) of Kansas City University, he received his M.D. in 1908 from the Long Island College of Medicine, Brooklyn, New York. He also did postgraduate work at Kansas University, St. Louis University, Chicago University, Rockefeller Institute for Medical Research and the Michael Reese and Cook County Hospitals in Chicago.

Dr. Matz entered the Government service in 1909 as Assistant Surgeon, National Military Home, Leavenworth, Kansas where he served as Chief of Laboratory. From 1914–1917 he conducted private laboratories in Kansas City and Leavenworth, Kansas and also served as consultant serologist to the Leavenworth Penitentiary.

In 1917 he was commissioned 1st Lieutenant M.C., U. S. Army and assigned as Chief of Laboratory Service, Camp Travis, Texas, being promoted to Captain in 1918.

After the War, Dr. Matz was commissioned Surgeon (Reserve) U. S. Public Health Service and served as Chief of Laboratory in various U. S. Public Health Service Hospitals until his appointment in 1925 as Chief of Medical Research in Central Office, which position he held at the time of his death.

A member of many medical organizations, Dr. Matz was well known as an investigator, administrator, and writer and as a physician and pathologist of skill and repute.

He died of coronary sclerosis, June 25, 1938 being survived by his Widow, Mother, three Brothers, and three Sisters. In his death the American Society

of Clinical Pathologists has lost one of its most valued members and clinical pathologists a colleague of distinction.

Dr. Samuel Walthall Budd, prominent Richmond pathologist, died at 12:30 A.M., July 27, 1938 at his home "Kingston," Chatham Hills, after an illness of several months.

Dr. Budd was taken ill last December with coronary thrombosis, but recovered sufficiently to take up his profession in March. However, on May 14th, he became ill again and was confined to his bed until the time of his death.

He was born in 1883 in Petersburg, Virginia. He attended preparatory schools in that city and later graduated from Hampden-Sydney College. He received his medical degree from Johns Hopkins University and took post-graduate work at the University of Freibourg, Germany. Later he served as interne at Johns Hopkins Hospital, and then as resident physician there. He also practiced in Petersburg and Norfolk before settling in Richmond. At one time he was a member of the faculty of the Medical College of Virginia, and had been a Vice-president of the American Society of Neo-Plastic Diseases.

Dr. Budd was considered one of the outstanding pathologists of this section and at the time of his death was pathologist for St. Luke's Hospital, the Retreat for the Sick Hospital, Grace Hospital, Petersburg Hospital and the Virginia Industrial Home for Girls.

EDITORIAL

The Medical Library Association*

Is your library in your institution, medical school, or county medical society a member of the Medical Library Association? Is the librarian of this library a professional member, and regular attendant at meetings? Are you a sustaining member, and do you know of the very interesting meetings that this association holds annually, and of the interesting papers on libraries and medical history that can be found only in the quarterly bulletin published by the Association?

It may seem strange to use the editorial pages of this Journal to extol the virtues of another society. However, no apologies are necessary on this score. These few remarks are not prompted by any suggestion from the Membership Committee and arise purely from the belief that enjoyment can be derived from membership in the Medical Library Association, which has been in existence now for forty years. The founder and first president was Gould, who was followed by Osler as president, and these in turn by Chadwick, Jacobi, Dock, Musser, C. Perry Fisher, J. C. Wilson, F. R. Packard, Lt. Col. McCulloch, H. L. Taylor, Browning, Garrison, Farlow, Barker, Wylde, Tice, Ruhräh, Malloch, Steiner, Miss Noyes, Frankenberger. Several of the above illustrious men served for three year terms. Dr. Francis of the Osler Memorial Library, was president from 1935 to 1937 and last year's meeting at Boston was presided over by Mr. Ballard of the Boston Medical Library who is President at this time.

If your library contains five hundred volumes, is open for regular use, and maintains a full time librarian it is eligible for membership in this Association. Such membership carries with it two votes by representatives sent from your library to

* Received for publication October, 1938.

attend meetings. Librarians also join as professional members. As individuals they have no votes. Physicians interested in medical libraries are more than welcome as sustaining members.

Probably the most important function of the association is the maintenance of the *Exchange*. Every member library has profited and has also rendered assistance by the exchange of duplicate material. Small libraries have often been able to complete very valuable files by this mutually helpful arrangement. The Medical Library Association is incorporated in the state of Maryland. It may receive bequests, donations, and endowments. But above all it merits the active support of the medical profession. Every pathologist should feel a keen interest in some local library. That library may be made better if he takes a real interest in the Medical Library Association, either by becoming a sustaining member, or by seeing to it that the library that he uses is a member.

A. H. Sanford

BOOK REVIEWS

Introduction to Physiological Chemistry. By MEYER BODANSKY, PH.D., M.D., Director of Laboratories, John Sealy Hospital, Galveston, and Professor of Pathological Chemistry, University of Texas. Cloth, Ed. 4, 686 pp., 41 figures, $4.00. John Wiley & Sons, Inc., New York.

When a book reaches a fourth edition it may be regarded as having established an accepted place in its field. Dr. Bodansky is well qualified, both by his standing as an investigator in the field of biochemistry and his experience as a teacher, to discuss authoritatively the subjects covered in this volume.

The present edition has been thoroughly revised and to a large extent rewritten so that it may be said to present adequately and in a very readable manner the present status of physiological chemistry with particular reference to its relation to clinical medicine.

This book may be recommended as a thorough, comprehensive and well written presentation. While written primarily for the student it may well and profitably be numbered among the reference volumes of the physician, pathologist and laboratory worker.

Taylor's Practice of Medicine. Edited by E. P. POULTON, M.A., D.M., F.R.C.P. (Lond.). Cloth, Ed. 15, 1136 pp., 104 figures, 71 plates, 16 in colors, $8.50. William Wood & Co., Baltimore.

That a book has reached a fifteenth edition is *ipso facto* evidence that it has amply fulfilled the purpose for which it was written. In the twenty-eight years which have elapsed since the first appearance this *Practice of Medicine* has become a standard text in England and has long been well and favorably known in this Country. This edition maintains the same standard as its predecessors and may be recommended as a comprehensive and authoritative reference text.

The Biology of Arteriosclerosis. By M. C. WINTERNITZ, M.D., R. M. THOMAS, M.D., and P. M. LE COMPTE, M.D. Cloth, 142 pp., 60 figures, in black and white and 56 colored plates, $4.00. Charles C. Thomas, Springfield, Ill.

This is a most stimulating study from the Department of Pathology of Yale University School of Medicine.

After commenting, in their introduction, upon the fact that arteriosclerosis has long been considered a disease *sui generis* the cause of which was lost in the mists of speculation, the authors emphasize that, after all, the vascular tissues are tissues and thus not exempt from the various primary pathological changes common to all tissues as a result of disease processes.

They have, therefore, made extensive studies of the blood supply of vessels

their disturbances, whether "adaptive," "physiologic" or pathological and have studied their effects and aftermath.

Their thesis is hence based on a recognition of the artery as a vascular or potentially vascular organ, therefore subject to the same pathological processes to which other tissues are subject. They thus open up and emphasize a fruitful field of inquiry.

It is not necessary to emphasize the standing of the authors and their fitness for the investigation they report.

It can be emphasized, however, that this book is well and clearly written, beautifully printed and magnificently illustrated. The black and white figures are excellently done and excellently reproduced but the color plates strike a new note in the illustration of American books. These are reproductions of Kodachrome microphotographs, in which the color shade and tones are remarkably "true to life," as it were.

This small volume contains much food for thought. Both authors and publisher deserve the highest commendation for its all around excellence.

Biological and Clinical Chemistry. By MATTHEW STEEL, PH.D., Professor of Biochemistry in the Long Island College of Medicine, Brooklyn, N. Y. Cloth, 770 pp., 15 figures, $8.00. Lea & Febiger, Phila., Pa.

This is a book intended primarily for the student, both as a teaching text and a laboratory manual.

While in the main, it will serve the purpose for which it is intended, a second edition will necessitate some revision, first, of the organization of the book as concerns the relative space allotted to the discussion of various phases of biochemistry, second, as regards errors not entirely typographical, and finally in the discussion of clinical applications. The references cited are not always the most recent.

In general the book will serve to call the attention of the student to the ever-growing importance of biochemistry in medicine and the necessity for some understanding of its varied phases by the physician.

Internships And Residencies. Report by The New York Committee on the Study of Hospital Internships and Residencies. Cloth, 492 pp., $2.50. The Commonwealth Fund.

For many a year it was the custom to lay great emphasis and to speak with feeling on what the interne owed the hospital, without, however, speaking nearly as often or with comparable emphasis upon what the hospital owed the interne. In this book is reported in full the results of a thorough and comprehensive study of the question by a joint Committee organized in 1934.

The interests of the investigation were focussed, in the main, on these questions:

Are internships and residencies properly correlated with the undergraduate curriculum? With graduate teaching?

Are they adequately related to the present day requirements of medical practice?

Do they offer continuing instruction and opportunity for thorough preparation for medical practice?

In what ways can internships and residencies be changed to improve the training of physicians?

This is a book which can be studied with profit by the Deans of Medical Schools, Hospital Administrators, Hospital Staffs, and indeed, all who have at heart the progress of medical education. This is a worth while study honestly evaluated and reported.

NEWS AND NOTICES

Revision of Constitution and By-Laws

As adopted at the meeting of the American Society of Clinical Pathologists, Cleveland, Ohio, June 10, 1934

CONSTITUTION

Article I—Name

This organization shall be known as the American Society of Clinical Pathologists.

Article II—Objects

The objects of this Society shall be: (a) To promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease; (b) to stimulate original research in all branches of clinical laboratory work; (c) to establish from time to time standards for the performance of various laboratory examinations; (d) to elevate the scientific and professional status of those specializing in this branch of medicine; (e) to encourage a closer coöperation between the practitioner and the clinical pathologist.

Article III—Membership

Section 1. The membership of this Society shall consist of (a) Fellows, (b) Associate, (c) Honorary, and (d) Corresponding Members.

Sec. 2. Fellows shall be graduates from recognized medical schools who have specialized in the practice or teaching of clinical pathology (the latter to be in a recognized medical school) for at least three years after graduation and who are devoting a major part of their time to this field. They shall be members in good standing of their county and/or state medical society or provincial medical society and of the American Medical Association or the Canadian Medical Society. For the purposes of this section Clinical Pathology shall be defined as that branch of the science and practice of medicine which consists of the application of pathologic anatomy, physiology, chemistry, parasitology and bacteriology to the diagnosis of disease.

Sec. 3. Associate members shall be graduates of recognized scientific institutions who have made such contributions to any of the sciences relating to clinical pathology and whose membership will so further the objects of the Society as to make them eligible for associate membership. Associate members

shall pay the regular dues and have all the privileges of Fellows except those of voting and holding office.

SEC. 4. Honorary members shall have distinguished themselves by research or personal sacrifice in the cause of scientific medicine to warrant their recommendation for election by the Board of Censors. They shall have all the privileges of active members except those of voting and holding office. They shall be exempt from paying dues.

SEC. 5. Corresponding members shall be residents of foreign countries in good ethical standing who have distinguished themselves in any of the branches of clinical pathology.

Article IV—Officers, Members of Standing Committees and Terms of Service

SECTION 1. The officers of the Society shall consist of a President, a Vice-President, a President-Elect and a Secretary-Treasurer. The President and Vice-President shall serve for one year. The President-Elect shall enter upon the duties of President at the annual meeting following his election. The Secretary-Treasurer shall serve for three years.

SEC. 2. The Standing Committees of the Society shall be an Executive Committee; a Board of Censors and a Board of Registry of Technicians.

SEC. 3. Officers and members of Standing Committees are to be proposed by the Nominating Committee or nominated from the floor by a Fellow of the Society and shall be elected by a majority of the votes cast at the annual business session.

SEC. 4. The Executive Committee shall be composed of six Fellows of the Society who shall each hold office for three years or until their successors are elected, two to be elected annually.

SEC. 5. The Board of Censors shall be composed of six Fellows of the Society who shall each hold office for three years or until their successors are elected, two to be elected annually.

SEC. 6. The Board of Registry of Technicians shall be composed of six Fellows who shall each hold office for three years or until their successors are elected, two of them to be elected annually.

SEC. 7. Officers and members of Standing Committees shall transfer promptly to their successors all funds, books, manuscripts, vouchers and other property of the Society on termination of their offices.

Article V—Meeting Place

The time and place of the annual meeting and other meetings of the Society shall be determined by the Executive Committee, notice of which shall be mailed to every Fellow at least thirty days prior to such meeting.

Article VI—Quorum

Twenty-five Fellows shall constitute a quorum.

Article VII—Amendments

This Constitution may be altered or amended by a vote of three-fourths of the Fellows voting at a regular meeting in executive session, provided said alteration or amendment had been submitted to the membership by publication or otherwise at least thirty days prior to the annual meeting.

BY-LAWS

Article I—Applications for Membership

Application for membership shall be made on a form authorized by the Society, signed by the applicant, recommended by two members and approved by the local Counselor and the Board of Censors. At least thirty days prior to the convention the Secretary shall send a list of applicants to every member of the Society.

Article II—Qualification for Membership

SECTION 1. Applicants for fellowship, associate and corresponding membership approved by the Board of Censors shall be elected by a Ballot of three-fourths of the Fellows voting at any regular meeting.

SEC. 2. Proposal for honorary membership may be made by a Fellow of the Society. Such proposal shall be made in writing and submitted to the Board of Censors. On recommendation by the Board such proposed member shall be elected as provided in Section 1.

Article III—Dues

SECTION 1. All Fellows, Associate Members, and Corresponding Members shall subscribe to this Constitution at the time of their election to membership and shall pay an initiation fee of Fifteen Dollars ($15.00), payable with the application for membership.

SEC. 2. The annual dues for Fellows, Associate Members, and Corresponding Members shall be Twelve Dollars ($12.00), payable December first for the following year and if unpaid on January first the subscription for the official Journal will lapse. Six Dollars ($6.00) of the annual dues shall be used as subscription for the official Journal. New members elected at the annual meetng shall pay dues for the current year of Six Dollars ($6.00) to cover the subscription of the entire volume of the official Journal for that year.

SEC. 3. Fellows in arrears for dues for sixty days shall be notified thereof by the Secretary-Treasurer by means of a "return receipt" registered letter. Fellows in arrears for ninety days shall be automatically dropped from the roll for non-payment of dues. Within one year after loss of membership for non-payment of dues, Fellows may be reinstated upon payment of all arrears and current dues.

SEC. 4. Resignation from the Society shall be submitted in writing to the

Secretary-Treasurer who shall cause the same to be presented to the Executive Committee at the next annual meeting and the resignation shall not become effective until acted upon by the Executive Committee. No resignation shall be accepted from a Fellow or member owing dues.

SEC. 5. An active member in good standing may become a life member by the payment of the sum as hereinafter stated or designated: up to forty-five years of age, Three Hundred Dollars ($300); forty-five to forty-nine years of age, Two Hundred and Eighty-Five Dollars ($285); fifty to fifty-four years of age, Two Hundred and Twenty-Five Dollars ($225); fifty-five years of age and thereafter, One Hundred and Fifty Dollars ($150).

Article IV—Duties of Officers and Standing Committees

SECTION 1. The President shall preside at all meetings of the Society. He shall appoint the Chairman of the Executive Committee and the Chairman of the Board of Censors, be an ex-officio member of all committees and perform all other duties that devolve on him by custom and parliamentary usage.

SEC. 2. In the absence from any meeting of the Society of the President, the President-Elect and in the absence of both, the Vice-President shall perform the duties of President.

SEC. 3. The President shall appoint the members of all Special Committees enumerated in Article V of these By-Laws except those of the Board of Registry of Technicians. He shall also appoint any additional special committees ordered by the Society and shall be empowered to appoint such others as he may consider necessary and for which he has secured the approval of a majority of the members of the Executive Committee.

SEC. 4. The Secretary-Treasurer shall keep a correct and permanent record of the meetings and the transactions of the Society. He shall furnish a copy of this record to the Editor of the official JOURNAL for publication, conduct the correspondence and perform such other duties as pertain to the office of Secretary. He shall receive and be the custodian of the funds of the Society except the funds of the Board of Registry of Technicians which shall be held by the Chairman of that Board. Within thirty days following the close of the annual meeting he shall present a budget for the ensuing year which shall meet with the approval of a majority of the members of the Executive Committee. He shall incur no additional expense during the year without the consent of a majority of the members of the Executive Committee. He shall give bond satisfactory to the Executive Committee, the cost of which shall be borne by the Society. He shall make a complete financial report at the annual meeting of the Society. He also shall be ex-officio Secretary of the Executive Committee.

SEC. 5. The Executive Committee shall be the executive and administrative body of the Society during the interval between the regular annual meetings and shall be empowered to enter into contracts and authorize such expenditures as may be necessary to carry on the affairs and the business of the Society. Its

actions always shall be governed by the Constitution and By-Laws of the Society. It shall audit the accounts of the Secretary-Treasurer as often as it deems necessary and the Chairman shall hold the bonds of the Secretary-Treasurer and the Chairman of the Board of Registry of Technicians. The Committee shall meet prior to the executive session of the Society. The Chairman shall prepare a report to be made to the executive session of the Society on its activities during the interval between the annual meetings and certify to the accounts of the Secretary-Treasurer and the Chairman of the Board of Registry of Technicians. The Committee shall meet also immediately after the annual meeting of the Society to transact such business as properly may come before it.

SEC. 6. The Board of Censors shall investigate all applications for membership and submit their recommendations at the annual meeting of the Society. They shall receive and consider all complaints concerning the conduct of members and present a report at the executive session with their recommendations. Suspension or expulsion from membership in the Society shall be by three-fourths vote of those members present and voting at a regular executive session.

SEC. 7. The Board of Registry of Technicians shall elect its own Chairman and Secretary. It shall conduct a Registry of Technicians, receive applications for such, pass on their qualifications and issue certificates and renewals of certificates to those meeting the requirements. It shall investigate schools for the training of technicians and register those approved. It shall conduct a placement bureau for technicians. Within thirty days following the close of the annual meeting the Chairman shall present a budget for the ensuing year which shall meet with the approval of a majority of the members of the Executive Committee. He shall incur no additional expense during the year without the consent of a majority of the members of the Board of Registry. The funds of the Registry shall be held by the Chairman of the Board who shall give bond satisfactory to the Executive Committee, the cost of which shall be borne by the Registry. The funds of the Registry shall be used only for the activities of the Registry. The Chairman shall make a complete financial report at the annual meeting of the Society.

Article V—Special Committees and Editor

SECTION 1. A Board of Counselors shall be appointed by the President to serve for one year. They shall represent such districts as may be determined by the President. It shall be the duty of the Counselors to act in the interest of the organization in their respective districts.

SEC. 2. A Nominating Committee of three Fellows shall be appointed by the President at the opening of the annual session, whose duty shall be to prepare a list of nominees for the elective offices for balloting by the Society. Additional nominations may be made from the floor.

SEC. 3. The President shall appoint a Program Committee consisting of three Fellows to serve for one year, the Chairman of which shall be the Secretary

of the Society, whose duty it shall be to arrange the scientific program for the annual meeting.

SEC. 4. The President shall appoint a Committee on Exhibits consisting of three Fellows, one of whom shall be the Secretary of the Society, to serve for one year, whose duty shall be to arrange for scientific and commercial exhibits at the annual meeting.

SEC. 5. The President shall appoint a Research Committee consisting of three Fellows, to serve for one year, whose duty it shall be to foster and direct collective investigation and to collect from rare or obscure conditions data and materials to be made available to Fellows for study.

SEC. 6. The Executive Committee shall appoint an Editor for the official JOURNAL of the Society to serve for a term of three years. The Editor so selected together with the President of the Society and the Chairman of the Executive Committee, shall appoint an Advisory Editorial Board to serve for a period of three years. The duties of this Board shall be to foster and supervise all official publications of the Society.

SEC. 7. The Executive Committee shall nominate members to serve on the Qualifying Board for Pathology in accordance with the stipulations of the by-laws of the American Board of Pathology.

Article VI—Awards

At each annual session the Research Committee may designate a Fellow of the Society to receive the Ward Burdick Award. This award shall be in the form of a gold medal which shall be presented to that Fellow who, in the opinion of the Research Committee, has presented the most meritorious contributions to the science of clinical pathology. Rules governing the award shall be made by the Research Committee, approved by the Executive Committee and published for the information of Fellows of the Society. If, in the opinion of the Research Committee, at any annual session no contribution is judged of sufficient merit to receive the award, no award shall be made at that session.

Article VII—Elections

SECTION 1. The Society shall elect annually by ballot at an executive session at the annual meeting the following officers and members of committees: President-Elect, Vice-President, two Fellows to fill vacancies on the Executive Committee, two Fellows to fill vacancies on the Board of Censors and two Fellows to fill vacancies on the Board of Registry of Technicians and such other vacancies as may have occurred. The Secretary-Treasurer shall be elected in the same manner each third year.

SEC. 2. Election shall be by a majority of votes cast by the Fellows present and shall be from nominees proposed by the Nominating Committee or from nomination made by any Fellow present.

SEC. 3. The President-Elect and newly-elected officers shall be inducted into office at the conclusion of the meeting.

Article VIII—Vacancies

In the event of a vacancy occurring in the office of President, the unexpired portion of his term of office shall be filled by the Vice-President. Vacancies occurring in the offices of Vice-President and Secretary-Treasurer shall be filled for the unexpired term of office by the Executive Committee. If a vacancy should occur in the office of President-Elect, at the next annual meeting the Society shall elect a President in addition to the officers enumerated in Article VII, Section 1 of the By-Laws. Interim vacancies occurring on the Executive Committee, the Board of Censors and the Board of Registry of Technicians shall be filled until the next annual meeting by the Executive Committee.

Article IX—Code of Ethics

SECTION 1. The Code of Ethics of this Society shall be the same as that of the American Medical Association.

SEC. 2. It shall be deemed unethical for members to publish objectionable laboratory advertisements in any form whatsoever; the Board of Censors shall act as judges in the matter, the members having the privilege of appeal to the Society at a regular executive session.

SEC. 3. It shall be considered unethical for a member to lend his name for publication in any laboratory advertisement or announcement which violates the Code of Ethics. The borrowing of names of other physicians, scientists or laymen, on the basis of an occasional service or consultation, for purposes of advertising or to sanction the work of a laboratory is misleading and unethical.

SEC. 4. Any system of dividing or rebating fees for laboratory services shall be considered unethical.

Article X—Gift and Bequest Fund

SECTION 1. There shall be established a gift and bequest fund for the following purposes: For publication expenses, to provide the dues of indigent members, for the purpose of research, and for other purposes as approved by the Executive Committee.

SEC. 2. This fund shall be administered by the Executive Committee.

Article XI—Standing Rules

SECTION 1. The Chairman, at all regular annual meetings, shall first call the members assembled to order in executive session for the purpose of transacting such business and appointing such committees as are herein required, together with the making of other arrangements consistent with conducting the annual meeting.

SEC. 2. Scientific papers presented by Fellows shall be limited to twenty minutes; those presented by guests shall not occupy more than thirty minutes. A longer time may be granted only by the consent of a majority of the Fellows present.

SEC. 3. The opening discussion on each paper shall be limited to ten minutes;

succeeding discussions shall be limited to five minutes each except as extension of time may be granted by a majority of the Fellows present.

SEC. 4. Members desiring to speak twice on any one subject must obtain the consent of a majority of the Fellows present.

SEC. 5. Non-members may be given the privilege of the floor only by consent of the majority of the Fellows present.

SEC. 6. A paper read before this Society becomes the property of the Society, to be published in the official JOURNAL provided it meets the approval of the Advisory Editorial Board, except that the privilege for prior publication may be granted by the Editor.

SEC. 7. Order of Business for Executive Session:

1. Call to order.
2. Reading of minutes.
3. Unfinished business.
4. Reports of committees.
5. Election of members.
6. New business.
7. Nominations.
8. Election of officers.
9. Induction of officers.
10. Adjournment.

Article XII—Parliamentary Procedure

All parliamentary proceedings at the meetings of this Society shall be governed by Roberts' Rules of Order, except where otherwise provided.

Article XIII—Amendments

Amendments of these By-Laws must be submitted in writing at the opening of the annual meeting and shall be voted upon at the executive business session. A majority of the votes cast shall be required to amend.

THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS ROSTER FOR 1938

Officers

Dr. T. B. Magath ..... President
Dr. W. Cummins ..... Vice-President
Dr. L. W. Larson ..... President-Elect
Dr. Alfred S. Giordano ..... Secretary-Treasurer

Executive Committee

Dr. R. R. Kracke, Chairman
Dr. C. W. Maynard
Dr. O. W. Lohr
Dr. W. M. Simpson
Dr. L. C. Todd
Dr. R. A. Kilduffe

Past Presidents

1922–3	Dr. Philip Hillkowitz	Denver, Colorado
1923–4	Dr. Wm. Carpenter MacCarty	Rochester, Minnesota
1924–5	Dr. John A. Kolmer	Bala-Cynwyd, Pa.
1925–6	Dr. Frederic E. Sondern	New York, N. Y.
1926–7	Dr. Wm. G. Exton	New York, N. Y.
1927–8	Dr. A. H. Sanford	Rochester, Minnesota
1928–9	Dr. F. W. Hartman	Detroit, Michigan
1929–30	Dr. J. H. Black	Dallas, Texas
1930–1	Dr. K. M. Lynch	Charleston, S. C.
1931–2	Dr. H. J. Corper	Denver, Colorado
1932–3	Dr. Walter M. Simpson	Dayton, Ohio
1933–4	Dr. Alvin G. Foord	Pasadena, California
1934–5	Dr. Frederick H. Lamb	Davenport, Iowa
1935–6	Dr. Foster M. Johns	New Orleans, La.
1935–6	Dr. R. A. Kilduffe	Atlantic City, N. J.
1936–7	Dr. R. R. Kracke	Emory University, Ga.
1937–8	Dr. C. W. Maynard	Pueblo, Colo.

Members of the American Society of Clinical Pathologists

Geographic Distribution

* Associate Members.
† Counselors.
§ Corresponding Members.
** Honorary Members.

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